UNIVERSITY
OF MICHIGAN
MAR 11 1958
SCIENCE

CANADIAN JOURNAL OF PLANT SCIENCE

VOLUME 37

January, April, July and October
1957

EDITED BY THE
AGRICULTURAL INSTITUTE OF CANADA
OTTAWA, CANADA

Published quarterly by the authority of the
Chairman of the National Committee on Agricultural Services

35-56784
subscription

946
8818
new

CANADIAN JOURNAL
OF PLANT SCIENCE

(formerly published as part of "*Canadian Journal of Agricultural Science*")

VOLUME 37 JANUARY, 1957 No. 1

STUDIES ON METHODS AND MATERIALS FOR TESTING ALFALFA FOR RESISTANCE TO BACTERIAL WILT[1]

M. W. CORMACK,[2] R. W. PEAKE AND R. K. DOWNEY[3]

[Received for publication July 4, 1956]

ABSTRACT

Several methods for inoculation of alfalfa with the bacterial wilt organism were compared in the greenhouse and field. The root-ball soak method, not previously described, proved particularly rapid and effective for greenhouse tests. All methods of root and crown inoculation tested gave good results, but spraying and other methods of stem inoculation were not dependable. In the field, the best results were obtained with bare-root soak inoculation before transplanting, and with hypodermic injection of the roots in the fall.

Cultures of the bacterium of tested virulence and infected plant materials proved equally effective as inoculum, but the latter were more easily handled and stored. The organism remained highly viable in dried plant material stored for three years at room temperature, 5° C., and −20° C. The pulverized plant inoculum gave best results when soaked in water for 4 to 12 hours before use. It was gradually inactivated with longer periods of soak.

Infection increased progressively with age in seedlings and rooted cuttings of Grimm alfalfa inoculated in the greenhouse at ages ranging from 3 to 10 weeks. Maximum susceptibility at 7 to 10 weeks of age was associated with the extensive formation of secondary tissues in the root and crown. The disease generally developed more rapidly in seedlings than in clonal material.

INTRODUCTION

Extensive programs for testing and breeding of alfalfa for resistance to bacterial wilt, caused by *Corynebacterium insidiosum* (McCull.) Jensen, have been in progress for many years in the United States (2, 4, 10, 12). Several methods of testing for resistance to this disease have been developed and successfully used by Jones (3, 4, 5, 6) and other workers. Little information has been published, however, on the relative effectiveness of different methods. For this reason, a comparative study of methods and materials was started in connection with a testing program at Lethbridge when the disease became prevalent in the irrigated areas of Alberta (9).

Preliminary to these studies, standards were established for the estimation of infection and damage caused by bacterial wilt. Various methods of inoculating alfalfa plants with the organism were studied in the greenhouse and field, and an attempt was made to develop a rapid

[1] Joint contribution from the Botany and Plant Pathology Division, Science Service (Contribution No. 1547), and the Forage Crops Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Officer-in-Charge, Plant Pathology Section, Science Service, Lethbridge, Alberta.

[3] Head, and Agrostologist, respectively, Forage Crops Section, Experimental Farm, Lethbridge, Alberta.

greenhouse method. Cultures and diseased plant materials were compared as sources of inoculum, and studies were made on storage and preparation of the plant inoculum. Information was also obtained on wilt infection in relation to age of plant in seedling and clonal material.

ESTIMATION OF INFECTION AND DAMAGE

Various methods of disease classification were used by previous workers to estimate resistance in alfalfa to bacterial wilt. Classes based on both root and top symptoms were as follows: Brink, Jones and Albrecht (1) and Peltier and Tysdal (10)—healthy, slightly infected, badly diseased, and dead; Donnelly (2)—free of symptoms, lightly infected, moderately infected, heavily infected, and dead. Wilson (13) described and illustrated four classes based on the degree of discoloration in the tap root. Simplified classes recently described by Jones (6) were: 1. non-infected; 2. with roots infected but showing no foliar symptoms; 3. showing foliar symptoms of infection or dead. This system has proved adequate for rapid classification in the screening of large numbers of seedlings.

In the present study, a fairly detailed and accurate measure was required of the degree of infection resulting from the different treatments. After tests were made with various classifications, six classes (symptoms illustrated in Figures 1 and 2) were chosen as follows:—

0. No infection.
1. Trace of root infection; small yellowish-brown spots or streaks visible on the wood when the bark of the tap root is peeled back.
2. Slight to moderate root infection, shown by more extensive discoloration which does not cover the entire surface of the woody cylinder and appears as spots or an incomplete ring in cross section; no top symptoms.
3. Severe root infection, shown by extensive discoloration which usually appears as a continuous ring in cross section; early top symptoms, consisting of stunted growth or of yellowing or wilting of the terminal leaflets.
4. Advanced top symptoms of severe dwarfing or wilting accompanied by deep root discoloration.
5. Dead or dying.

The data were recorded as number of plants in each of these classes, and the average numerical disease rating was calculated for each variety or treatment. The percentages of plants that were disease free (class 0), with root symptoms only (classes 1-2), with top symptoms (classes 3-5), and severely damaged or dead (classes 4-5), could also be determined from these data. In some experiments the first appearance of top symptoms and the rapidity of killing of the plants were also recorded.

METHODS OF INOCULATION

Several methods of inoculating alfalfa plants with the bacterial wilt organism have been previously described. Jones (3) developed the first adequate and practicable method. The plants were inoculated at the time of transplanting to the field by scraping the roots lightly with a knife under a bacterial suspension prepared from pure cultures. In later tests, Jones (4) cut the roots under a bacterial suspension and also re-inoculated the plants that remained healthy in the field by inserting inoculum in a slit in each root. The need for this second inoculation to

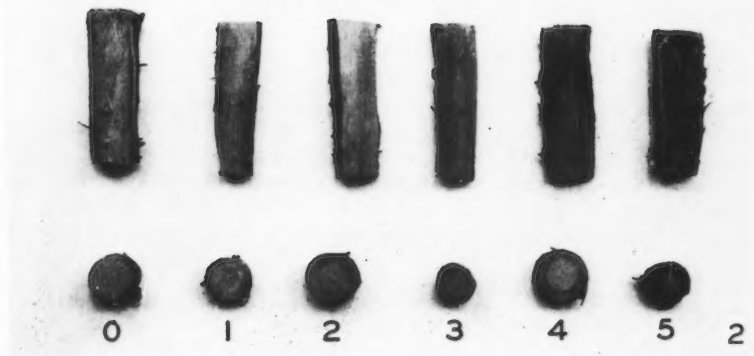

FIGURE 1. Alfalfa plants infected to varying degrees by bacterial wilt. *Left to right:* No top symptoms (classes 0—2); early top symptoms (class 3); advanced top symptoms (class 4).

FIGURE 2. Root sections of alfalfa plants illustrating progressive degrees of infection with bacterial wilt (classes 0—5). *Top row*—Bark peeled back. *Bottom row*—Cross section.

eliminate all susceptible plants in field tests was demonstrated by Jones (5) and Peltier and Tysdal (10). Other workers (2, 12, 13) immersed the cut roots in a bacterial suspension prior to planting in the field or greenhouse. Donnelly (2) kept the plants under a partial vacuum for 30 minutes while the roots were immersed, and re-inoculated the surviving plants by hypodermic injection into the tap root or by clipping off the tops with shears dipped frequently in a bacterial suspension.

The inoculation methods compared in this study included various modifications of those used by other workers. These methods will be briefly described and discussed before the results of the comparative studies are presented.

Inoculation of Single Roots

The root-wound insert method involved placing the inoculum in a slit made with a sharp knife slightly below the crown in each root. Small portions of a bacterial suspension or of infected plant material were used as inoculum. This method was used extensively for the uniform inoculation of plants for inoculum tests and other experiments, but it was too laborious for general use. It was more easily applied to lifted plants before transplanting than to those growing in soil.

Hypodermic injection or needle inoculation of individual roots or crowns with a bacterial suspension was also very time-consuming. A large needle (No. 19 or 20) attached to a rubber bulb reservoir was used in most experiments. Difficulty was experienced with plugging of the hole in the needle and with regulation of the quantity of inoculum applied, especially with plants growing in the field. A side-opening needle supplied by F. R. Jones gave the best results.

Bare-Root Soak

This method, named the bare-root soak to differentiate it from the other methods described, is similar to the dip or immersion methods used by several other workers (2, 12, 13). Plants grown in the field or greenhouse were lifted, the roots cut off about 4 inches below the crowns and immediately placed in a bacterial suspension. After soaking for at least one-half hour the plants were transplanted into field plots or greenhouse pots. Mass handling of the plants by this method was more rapid and convenient than inoculation of single roots, but there was a marked set-back in growth after transplanting.

Root-Ball Soak

This previously undescribed method has proved to be very rapid and effective in greenhouse experiments. Alfalfa plants about 3 months old with a good mass of roots (10 to 20 plants per 6-inch pot), were thoroughly watered shortly before inoculation. The moist mass of roots and soil (root-ball) was knocked out of each pot on a tray or board. This root-ball was cut through with a large knife about 2 inches below the soil surface so that the main roots of all plants in the pot were severed. The lower portion of the root-ball was immediately replaced in the pot and was submerged in inoculum by pouring a measured quantity (usually 100 c.c.) of a bacterial suspension prepared from cultures or infected plant material. The upper portion was then replaced so that the cut ends of

the roots came in contact with the inoculum. It was possible to perform this treatment rapidly and uniformly, and there was no noticeable disturbance of growth of the plants. A new mass of roots developed at the cut ends of the tap roots and usually refilled the bottom portion of the pots within two weeks after inoculation.

Crown Soak

Attempts were made to develop a simple method of crown inoculation that would eliminate disturbance of the roots. In the greenhouse tests, a measured quantity of bacterial suspension was poured into each pot after the crowns of all plants had been wounded with a sharp knife or needle. The chief limitations of this method were the time required to wound all crowns uniformly and the danger of causing serious injury to small plants. The total time required for wounding of the crowns and inoculation was much greater than for the root-ball soak method, and wounding of the plants was usually less uniform.

Inoculation of Stems

Inoculation of alfalfa stems was attempted by several methods including cutting with a knife dipped frequently in a bacterial suspension (8) and spraying of the plants with inoculum after the tops were cut. These rapid and convenient methods unfortunately allowed many plants to escape infection and did not give dependable results.

COMPARATIVE INOCULATION TESTS

Greenhouse Tests

Methods of inoculation were compared in several greenhouse experiments. Plants of Grimm alfalfa, 3 to 6 months old, grown in pots (15 to 20 plants per pot) or transplanted from the field (5 plants per pot) were inoculated with a bacterial suspension prepared from fresh cultures. Six replicates of 6-inch pots were used for each treatment. To encourage disease development the plants were well supplied with water in a warm greenhouse, and the tops were cut back at monthly intervals. The first

TABLE 1.—DEVELOPMENT OF BACTERIAL WILT IN GRIMM ALFALFA PLANTS 3 MONTHS AFTER INOCULATION BY DIFFERENT METHODS IN THE GREENHOUSE (AVERAGE RESULTS OF 5 EXPERIMENTS)

Method of inoculation	Percentage of diseased plants			Average disease rating
	Root symptoms only	Top symptoms or death	Total	
Root-wound insert	38	59	97	2.9
Bare-root soak	32	63	95	2.7
Root-ball soak	35	61	96	2.6
Crown soak	35	60	95	2.5
Hypodermic—root	45	52	97	2.3
Hypodermic—crown	37	53	90	2.1
Hypodermic—stem	37	36	73	1.9
Stem inoculation (knife)	41	32	73	1.5
Stem inoculation (spray)	22	39	61	1.5
Unwounded—inoculated	10	10	20	0.5
Unwounded—not inoculated	2	0	2	0.1

top symptoms of bacterial wilt usually appeared within 6 weeks. All plants were taken up and rated for disease development 3 months after inoculation.

As shown in Table 1, the average results of five experiments indicated that all the methods of root inoculation tested were highly effective. The crown soak also gave good results, but the degree of infection was slightly lower with hypodermic injection in the crown than in the root. The importance of artificial wounding was shown by the low percentage of infection that occurred when a bacterial suspension was poured in pots of unwounded plants. Infection was very slight in the non-inoculated control plants.

Stem inoculation resulted in a generally lower percentage of infected plants than root and crown inoculation. Hypodermic injection was less effective in the stem than in the root or crown. The convenient method of spray inoculation of the cut stems was the least dependable of the methods tested. In another experiment, infection was further reduced when the spray application was delayed 3 hours after the plants were cut. The relatively low degree of infection resulting from stem inoculation may have been caused by slow or variable absorption and conduction of the bacteria in the stems (8).

Field Tests

Several experiments were conducted to determine the most suitable methods of inoculation for field conditions. Most of the methods studied in the greenhouse were used to inoculate alfalfa plants *in situ* or at transplanting time in the field. As in the greenhouse, spraying of the tops with inoculum and other methods of stem inoculation proved relatively ineffective and were discontinued.

A comparison was made of the Grimm, Ladak and Ranger varieties inoculated by several promising methods in a split plot experiment in which varieties constituted the main plots and methods the sub-plots. About 600 plants of each variety, in 6 replicates, were inoculated by the bare-root soak method (plants dug up and re-planted); and *in situ* by hypodermic injection in the fall and spring, and by the application of a bacterial suspension to the freshly wounded crowns and roots. In the root-soak method, the tap roots were cut off about 3 inches below the crown and inoculum was poured in before the spade was withdrawn, in an attempt to simulate the root-ball soak method used in the greenhouse. The control plants were not artificially wounded or inoculated.

The bare-root soak method of inoculation produced the highest degree of infection in all 3 varieties tested (Table 2). Hypodermic injection in the fall also resulted in a significantly higher percentage of diseased plants and infection rating in Grimm and Ladak than did a similar inoculation in the spring. Rather variable results were obtained with the root- and crown-soak methods and they appeared to be impractical for field use.

With all methods tested, Grimm proved relatively susceptible; Ladak, moderately resistant; and Ranger, highly resistant. These differences were most evident in the results obtained with the best methods of

TABLE 2.—DEVELOPMENT OF BACTERIAL WILT IN 3 VARIETIES OF ALFALFA 2 YEARS AFTER INOCULATION BY DIFFERENT METHODS IN THE FIELD

Method of inoculation	Percentage of diseased plants			Transformed value average[1]			
	Grimm	Ladak	Ranger	Grimm	Ladak	Ranger	Average
Bare-root soak	94	67	45	79	56	43	59
Hypodermic—fall	88	65	35	71	55	36	54
Hypodermic—spring	52	36	14	46	37	22	35
Cut-root soak	66	43	28	56	41	34	44
Crown soak	73	41	23	59	39	30	43
Control	28	24	12	32	28	19	26

[1] Angle transformation of percentage values as given by Snedecor (11)
Least significant difference (1% level):
Between two method means within one variety = 15
Between two variety means with same method = 15
Between two methods on a single plot basis = 9

inoculation. A relatively low degree of natural infection occurred in the non-inoculated control plants.

In large-scale field experiments, it is sometimes necessary or desirable to store inoculated plants for a short time before they can be planted. To determine the effects of storage, plants inoculated by the bare-root soak method were held under moist conditions for various periods at room temperature and at 5° C. The plants stored at room temperature for 3 days, and at 5° C. for 7 days grew normally and had as high a degree of infection as those that were planted immediately after inoculation.

STUDIES ON INOCULUM

Most workers have used bacterial suspensions prepared from pure cultures for inoculation of alfalfa plants with the wilt organism (2, 4, 12). Jones (3) obtained good results with a combination of infected root fragments dipped in a bacterial suspension. Kernkamp and Hemerick (7) recently reported better results with inoculum prepared from infected alfalfa roots than from cultures.

Comparison of Cultures and Plant Inoculum

Bacterial suspensions were prepared by brushing and washing the growth from agar slant cultures about 1 week old, or by mixing the entire contents of each tube in a Waring blendor. Dilutions ranging from 100 to 500 cc. of water for each tube proved satisfactory. Addition of aerosol (0.05 per cent) to the suspension did not increase the degree of infection obtained by soak or spray inoculation. Most cultures tested proved highly virulent but there were some exceptions (Table 3). Certain isolates also proved unstable in culture and tended to lose their virulence. In practice, new isolates or re-isolates, obtained at least yearly, were tested for virulence in the greenhouse before they were used for large scale tests.

Alfalfa plants infected with bacterial wilt were used for plant inoculum. These plants were dried at room temperature and pulverized in a Wiley Mill with a screen mesh of 0.5 mm. The results of inoculation tests with plant material infected to varying degrees showed that the inoculum

TABLE 3.—EFFECT OF KIND OF INOCULUM AND STORAGE ON DEVELOPMENT OF BACTERIAL WILT IN GRIMM ALFALFA IN GREENHOUSE INOCULATION TEST.

Inoculum	Storage[1]	Total diseased plants		Average disease rating
		%	Transformed value average[2]	
Culture No. 1		98	87	2.9
Culture No. 2		94	82	2.4
Culture No. 3		79	63	2.2
Diseased plants	Dry at room temperature	89	74	2.7
Diseased plants	Dry at 5° C.	95	83	3.0
Diseased plants	Dry at −20° C.	92	78	2.9
Diseased plants	Fresh at −20° C.	85	72	2.2
Least significant difference (1% level)			14	0.7

[1] Stored for 3 years under the conditions indicated.
[2] Angle transformation of percentage values as given by Snedecor (11).

was highly infective when prepared from the roots or tops of moderately to severely diseased plants with the symptoms described for classes 3 to 5 (Figure 2). Before use, this inoculum was soaked in water (1 gram to 100 cc.) for about 4 hours.

In comparative tests of culture and plant inoculum, healthy plants of Grimm alfalfa were inoculated in the greenhouse by the root-wound insert or root-ball soak method. Neither type of inoculum proved consistently superior as measured by the percentage of infected plants or average infection rating. As shown in a representative experiment (Table 3), there were no significant differences in the results obtained with virulent cultures and dry diseased plant material.

Storage of Plant Inoculum

One advantage of plant inoculum is that it can be readily stored for future use. Kernkamp and Hemerick (7) described a "deep freeze" method of preservation and found that infected alfalfa roots stored at −20° C. for 21 months caused moderate to severe infection in 6 weeks in a greenhouse inoculation test. In comparative tests with this method of storage and others (Table 3), the dried plant material gave equally good results when stored at room temperature, 5° C. and −20° C. for 3 years. Fresh material, frozen without being dried, and stored at −20° C. gave a slightly lower infection rating. In other experiments, the pulverized material prepared from diseased plants, and the dried but unpulverized plants stored at room temperature, 5° C. and −20° C. for 3 years, proved as infective as freshly collected plant material.

Preparation of Plant Inoculum

The period of soak in water proved important in obtaining good results with inoculum prepared from diseased plants. Maceration in water of the pulverized or shredded material was necessary to allow maximum diffusion of the bacteria from the tissues. In a preliminary test, plant inoculum soaked in water for 4 hours gave good results, but the same material soaked for 20 hours was completely inactivated. Other lots of

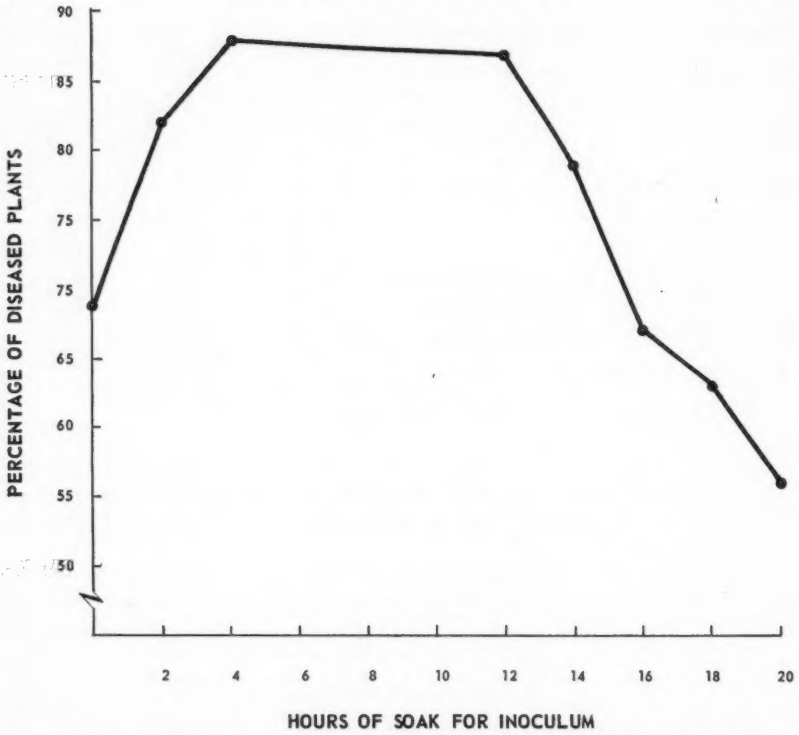

FIGURE 3. Effect of period of water soak for plant inoculum on development of bacterial wilt in Grimm alfalfa.

inoculum showed the same general trend when soaked in tap or distilled water but some were less severely affected than others by a long period of soak. In one experiment, portions of the inoculum were used immediately after it was placed in tap water and after periods of 2, 4, 12, 16, 18 and 20 hours of soak. As shown in Figure 3, the material caused increased infection with 2 and 4 hours of soak but decreased progressively in infectivity after 12 hours in water. Bacterial suspensions prepared from cultures, however, remained highly viable after 20 hours. These results indicate that the inactivation of the bacteria in water may have resulted from the antibiotic effect of other organisms or from other factors associated with the plant material.

INFLUENCE OF AGE AND TYPE OF PLANT

Jones (5) found that very young alfalfa seedlings were apparently immune to bacterial wilt and that the plants had a greater chance of escaping infection when one month old than at a later stage. To obtain more accurate information on the stage at which the plants can be successfully inoculated, a test was made with seedlings and rooted cuttings ranging in age from 3 to 10 weeks. The material was grown in the greenhouse by

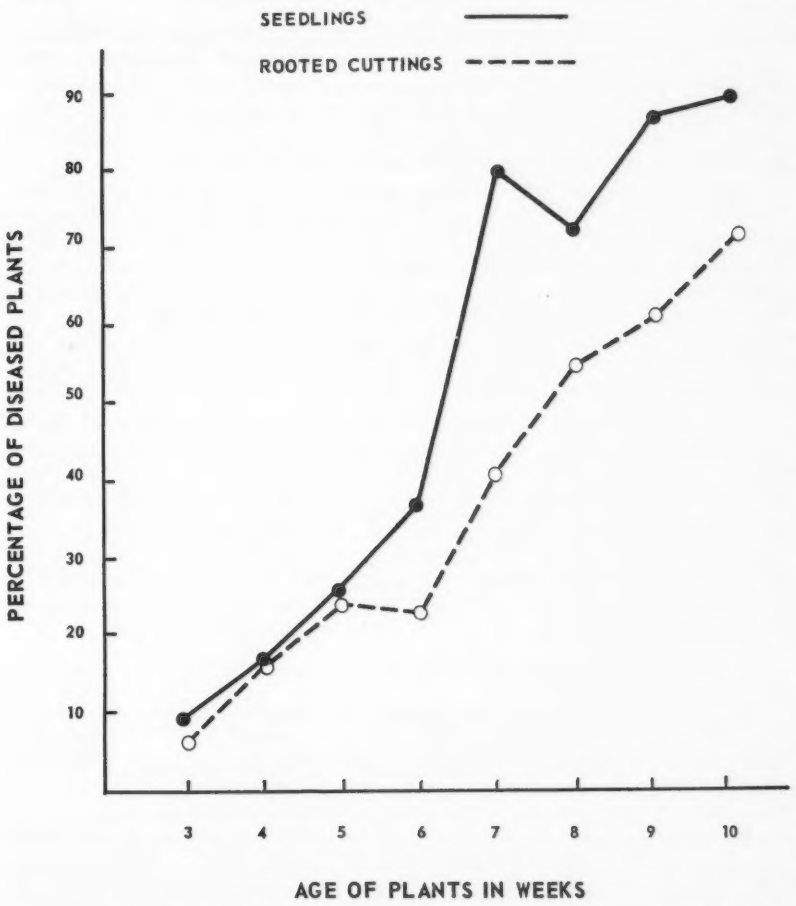

FIGURE 4. Development of bacterial wilt in seedlings and rooted cuttings of Grimm alfalfa inoculated at different ages in the greenhouse.

planting seeds and starting cuttings of Grimm alfalfa at weekly intervals for 8 weeks. In computing age, one week was allowed for seed germination and for initiation of roots in the cuttings. The material of all ages was inoculated at one time by the root-ball soak and crown soak methods (5 replicate pots each). At this time, root and crown material from plants of all ages was also preserved for anatomical study.

Similar results were obtained with the root and crown methods of inoculation, but the general level of infection was lower with crown inoculation. As shown by the results of the root inoculation (Figure 4), slight infection occurred in 9 per cent of the seedlings and 6 per cent of the rooted cuttings inoculated at 3 weeks of age. Susceptibility to the bacterial wilt organism, indicated by percentage of diseased plants, increased

progressively with age of plant. In the seedlings the greatest difference occurred between plants inoculated at 6 and 7 weeks of age where there was an increase from 37 to 80 per cent of diseased plants. There was a further increase to 90 per cent in the plants inoculated at 10 weeks of age. Susceptibility increased more slowly in the rooted cuttings of which 61 per cent were diseased after inoculation at 10 weeks of age.

Anatomical studies on alfalfa seedlings of the same ages as those inoculated showed that the primary tissues of the root and crown remained at least partly functional until the plants were 6 weeks old. At this time there was a pronounced development of secondary vascular tissues which almost completely superseded the primary tissues in 7-week-old seedlings. Since susceptibility to bacterial wilt also increased rapidly at this age (Figure 4), these results confirm the association, suggested by Jones (5), between liability to infection by the organism and the formation of secondary tissues.

The relative susceptibility of seedlings and rooted cuttings of alfalfa was studied in several experiments. Infection usually developed more slowly in young rooted cuttings than in seedlings (Figure 4), but this difference was less evident in older material. In one greenhouse test, the percentages of diseased plants in 3-month-old seedlings and rooted cuttings respectively, were 98 and 86 per cent in Grimm, 63 and 61 in Ladak and 28 and 17 in Ranger. These results show that the relative susceptibility of alfalfa varieties to bacterial wilt is the same when either seedling or clonal material is tested.

DISCUSSION AND CONCLUSIONS

These results indicate that several methods of inoculation can be successfully used in testing alfalfa for resistance to bacterial wilt. Any method that places the organism in fresh wounds in the root or crown should be effective if conditions are favourable for disease development. The method chosen may depend on the purpose, location and time of the test, and on the type and quantity of material to be tested. Convenience and rapidity are also important factors, especially in large scale tests. For greenhouse tests, the root-ball soak method appears to be most suitable, since large numbers of plants can be rapidly and uniformly inoculated without individual handling or disturbance of growth. Under field conditions, the best results were obtained with bare-root soak inoculation at transplanting time, and hypodermic injection of the roots in the fall. These two methods can be combined for rigid elimination of susceptible plants.

Inoculum prepared from diseased plants has proved as effective and practical as culture inoculum. Plant inoculum has the distinct advantage that it can be stored for a long period under various conditions without loss of viability. Also, it automatically provides a mixture of all strains of the organism that may be present in the diseased material. Necessary precautions are the careful selection of diseased plants to be used as inoculum, and a limited period of water soak for the pulverized material so that the bacteria will not be inactivated. Cultures of tested virulence are superior to plant material if a highly standardized inoculum is required. A mixture of several isolates is desirable to provide different

possible strains of the organism. The chief disadvantage of cultures is the care required to maintain them in a virulent condition.

The age and type of plant should also be considered in tests for resistance to bacterial wilt. Alfalfa seedlings should be at least 2 months old before inoculation, while rooted cuttings may require a somewhat longer period to become susceptible. The disease usually develops more rapidly in seedlings than in rooted cuttings. Clonal material, however, is quite suitable and is required in some tests.

ACKNOWLEDGEMENTS

The writers wish to express their appreciation to J. E. Moffatt and E. J. Hawn, Plant Pathology Laboratory, Lethbridge, Alberta, for assistance with many of the experiments, and to acknowledge the advice of the late F. R. Jones, University of Wisconsin, during this study.

REFERENCES

1. Brink, R. A., F. R. Jones, and H. R. Albrecht. Genetics of resistance to bacterial wilt in alfalfa. J. Agr. Research 49: 635-642. 1934.
2. Donnelly, E. D. Screening for wilt resistance in alfalfa. Agron. J. 44: 386-387. 1952.
3. Jones, F. R. Bacterial wilt of alfalfa. J. Am. Soc. Agron. 22: 568-572. 1930.
4. Jones, F. R. Testing alfalfa for resistance to bacterial wilt. J. Agr. Research 48: 1085-1098. 1934.
5. Jones, F. R. Artificial inoculation of alfalfa to determine susceptibility to bacterial wilt. *In* Rept. 8th Alfalfa Imp. Conf.: 10-14. 1940.
6. Jones, F. R. Bacterial wilt. *In* Rept. 13th Alfalfa Imp. Conf.: 54. 1952.
7. Kernkamp, M. F., and Glen Hemerick. A "deep-freeze" method of maintaining virulent inoculum of the alfalfa wilt bacterium, *Corynebacterium insidiosum*. (*Abstract*). Phytopathology 42: 13. 1952.
8. Koehler, B., and F. R. Jones. Alfalfa wilt as influenced by soil temperature and soil moisture. Illinois Agr. Expt. Sta. Bull. 378. 1932.
9. Peake, R. W., and M. W. Cormack. Effect of bacterial wilt on hay yield of irrigated alfalfa. Can. J. Agr. Sci. 35: 202-210. 1955.
10. Peltier, G. L., and H. M. Tysdal. Wilt and cold resistance of self-fertilized lines of alfalfa. Nebraska Agr. Expt. Sta. Research Bull. 76. 1934.
11. Snedecor, G. W. Statistical methods. 4th ed. Iowa State College Press, Ames, Iowa. 1946.
12. Weimer, J. L., and B. A. Madson. Relative resistance to bacterial wilt of certain commercial and selected lots of alfalfa. J. Agr. Research 52: 547-555. 1936.
13. Wilson, M. C., Jr. Inheritance of resistance in alfalfa to bacterial wilt. J. Am. Soc. Agron. 39: 570-583. 1947.

DISCRIMINANT FUNCTION ANALYSIS IN IDENTIFICATION OF PEACH VARIETIES IN NURSERY TREES[1]

K. LAPINS[2] AND S. W. NASH[3]

[Received for publication June 11, 1956]

ABSTRACT

Discriminant function analysis was applied to test the possibility of identifying five peach varieties from nursery trees which had been measured for five leaf characteristics. Variation of the five leaf characters was found to be mainly in two dimensions and was expressed by two discriminant functions. The compound scores of two discriminant functions of the varieties could be simply plotted on two axes of a chart. Application of the discriminant functions is simple and consists in comparing the compound scores of an unknown sample to those of the average values of known varieties by plotting them on the chart. Individual 20-leaf samples, when plotted on the chart, were found to scatter rather widely and the points representing related varieties frequently overlapped. However, using variety Valiant as standard and adjusting the variation caused by location effect, variation was reduced to such an extent that application of discriminant functions in peach variety identification could be considered practicable.

INTRODUCTION

Leaf measurements were used as a means of identification of peach varieties in the variety studies in commercial fruit tree nurseries in British Columbia (7). Measurements of 20-leaf samples usually gave significant differences between varieties *at each location*, but the average values of different varieties frequently overlapped when samples *from different locations or years* were compared, thus making identification unreliable if varieties were observed in only one character at a time.

Discriminant function analysis can be employed as a means of comparing varieties in all the measured characters simultaneously. A set of multiple measurements is used to construct a discriminant function which has the property that it will discriminate better than any other linear function between the two or more classes. Fisher (4) in 1936 introduced the discriminant function analysis for separating two or more groups of individuals in which several characters had been measured. Since then discriminant functions have found application in various fields of research where differentiation between the classes is required. Besse Day and Marion Sandomire (2) used discriminant functions of several deer measurements as a means of determining the age of animals. Smith (12), Panse (9) and Goulden (6) showed how discriminant functions can be applied in breeding work. Rao and Slater (11) classified mentally ill officers of the Army and Navy into several neurotic groups on a basis of several characters observed, and this publication provides a good example of the use of the discriminant functions for more than two groups. The theory and technique of application of discriminant functions in separating individuals into two groups is described by Fisher (5) and Goulden (6). Rao (10) deals with separation into two or more groups.

[1]Joint contribution No. 874 from Horticulture Division, Experimental Farms Service, Department of Agriculture, Ottawa, and Department of Mathematics, University of British Columbia, Vancouver, B.C.

[2]Agricultural Research Officer, Canada Department of Agriculture, Summerland, B.C.

[3]Assistant Professor of Mathematics, University of British Columbia, Vancouver, B.C.

The present report shows how discriminant functions are calculated and applied to a popular and practically important problem in horticulture—the identification of peach varieties from nursery trees.

PROCEDURE AND RESULTS

Five peach varieties—Elberta, Spotlight, Valiant, Vedette and Veteran—were measured for five leaf characters: length of leaf blade, width of leaf blade, base angle, apex angle and length of petiole. Leaf samples were collected in 5 commercial fruit tree nurseries, representing diverse climatic and soil conditions, and the experiment was continued for 3 years. A sample consisted of 20 leaves, collected from 20 trees and from a common and definite zone of the tree.

In the present problem discriminant functions should aid in deciding to which variety an unknown sample belongs, when the sample has been measured for the five characters. A discriminant function can be set up as follows:

$$z = k_1x_1 + k_2x_2 + k_3x_3 + k_4x_4 + k_5x_5 ,$$

where $x_1, \ldots, x_5$ are the mean values of the five characters measured, and $k_1, \ldots, k_5$ are the weights of the individual characters. The coefficients are chosen in such a way that the values of z for one group will differ as much as possible from the values of z for another group.

Fisher (4) has shown how to calculate the coefficients $k_1, k_2, \ldots, k_p$ of the discriminant function, if just two groups are to be discriminated. For simplicity we take p, the number of characters, to be three. Let $w_{ij} = \Sigma(x_i - \bar{x}_i)(x_j - \bar{x}_j)$, the within groups sum of products for the i-th and j-th characters, and let d_1, d_2, d_3 designate the differences of the group means for each of the characters. The coefficients required are proportional to the solutions of the equations

$$k_1w_{11} + k_2w_{12} + k_3w_{13} = d_1$$

$$k_1w_{12} + k_2w_{22} + k_3w_{23} = d_2$$

$$k_1w_{13} + k_2w_{23} + k_3w_{33} = d_3 .$$

There are several simple routine procedures for solving a set of such equations, for example, the abbreviated Doolittle procedure, which can be found in Goulden (6) and many other references.

The problem is more complicated if several groups—five in the present case—are to be separated. However, the difference in the algebraic solutions between the general case with several groups and the simple case with two groups is more apparent than real, for the two-group problem allows simplifications of the formulas not possible in the general case. Rao and Slater (11) compared six groups of individuals and exemplified a method of calculating the discriminant functions, and this procedure is mainly followed for the present problem.

TABLE 1.—PARTITION OF COVARIATION INTO COMPONENTS OF THE EFFECTS

Source of covariation	D.F.	Covariation between									
		Length and width	Length and base angle	Length and apex angle	Length and petiole	Width and base angle	Width and apex angle	Width and petiole	Base angle and apex angle	Base angle and petiole	Apex angle and petiole
		Sums of products (S.P.)									
Total	68	−70.9449	−154.1039	−51.2868	8.4064	1150.0594	341.2984	−69.7092	840.6387	−217.1146	−55.9275
Varieties (V)	4	−51.3943	−78.2319	−43.9613	−0.0389	905.7257	207.1173	−46.8784	478.9720	−189.7714	−15.8742
Locations (L)	4	−17.3987	−72.0219	−16.0679	6.3647	151.6617	109.2387	−27.4217	200.3020	−56.3205	−45.8075
Years (Y)	2	1.7705	−54.2436	−3.3812	−0.1904	8.7388	1.8392	1.0831	80.3755	12.4834	1.1241
V x L	16	−2.0238	−1.0724	−0.8399	−1.0808	16.7223	5.3061	1.2934	11.7847	0.2343	1.1855
V x Y	8	−1.2671	−1.8940	−0.5359	0.4351	9.4617	0.2313	−1.1200	4.4912	−1.7400	0.2868
L x Y	8	1.7624	54.6878	12.9993	2.4656	38.7513	9.3509	2.7958	55.5992	18.4505	1.8437
V x L x Y	26	−2.3939	−1.3279	0.5001	0.4511	18.9979	8.2149	0.5386	9.1141	−0.4509	1.3141
		Correlation coefficients (r)									
Between varieties	—	−0.688	−0.346	−0.855*	−0.003	0.913*	0.918**	−0.671	0.702	−0.898*	−0.330
Within varieties	—	−0.388*	−0.083	0.066	0.179	0.614**	0.557**	0.110	0.239	−0.036	0.219

Let b_{ij} denote the sum of products between varieties for the i-th and j-th leaf characters. Let w_{ij} denote the corresponding sum of products within varieties. The b_{ij} are the between variety sums of squares and products occurring in ordinary analysis of variance and covariance; the w_{ij} are the corresponding error sums, in the present report the variety-location-year interaction sums. If we arrange the numbers b_{ij} in rectangular fashion so that b_{ij} appears in the i-th row and j-th column, the resulting array is called a matrix.

We let

$$B = \text{sum of products matrix between varieties}$$

$$W = \text{sum of products matrix within varieties.}$$

The basic formula for calculation of the discriminant functions requires the solution for λ of the equation

$$|B - \lambda W| = 0 ,$$

where the left side is the determinant of the matrix $B - \lambda W$, whose element in the i-th row and j-th column is $b_{ij} - \lambda w_{ij}$. Letting W^{-1} denote the inverse matrix to W, and λ denote a matrix with the number λ down the principal diagonal and zeros elsewhere, the previous equation is equivalent to the equation

$$|BW^{-1} - \lambda| = 0 .$$

This is a polynomial in λ. Procedures for calculating inverse matrices, products of matrices, and the characteristic polynomial above are given by Dwyer (3) and others.

The five measurements made on a peach leaf or leaf average can be conceived of as defining a space of five dimensions. The discriminant functions replace the original measurements by new coordinates which are appropriate when a new set of coordinate axes has been chosen in such a way that as much of the distances between variety means as possible is accounted for by the direction of the first new coordinate axis, as much as possible of the remaining differences between variety means is accounted for by the direction of the second coordinate axis, and so on. The points belonging to one variety cluster about the mean value of the variety more or less in the shape of an ellipsoid. Strictly speaking, the points representing the variety means need not form an ellipsoidal cluster about a common center, but at least their relative degree of scatter in different directions can be represented by the lengths of lines in those directions from the center to the outer surface of such an ellipsoid. Matrix B gives information as to the lengths and directions of the principal axes of this ellipsoid. Use of BW^{-1} instead of B brings all measurements into standard units. Hence the final results will be unaffected by whether the original measurements were in inches or centimetres, etc. The problem is to calculate the directions of the various axes of this ellipsoid. These become the new coordinate axes, and the discriminant functions give us the new coordinates which replace the original observations.

TABLE 2.—S.S. AND S.P. MATRIX WITHIN VARIETIES OR VLY MATRIX (W)

	x_1	x_2	x_3	x_4	x_5
x_1	3.1768	−2.3939	−1.3279	0.5001	0.4511
x_2	−2.3939	11.9899	18.9979	8.2149	0.5386
x_3	−1.3279	18.9979	79.9649	9.1141	−0.4509
x_4	0.5001	8.2149	9.1141	18.1046	1.3141
x_5	0.4511	0.5386	−0.4509	1.3141	1.9979

TABLE 3.—INVERSE MATRIX OF VLY (W^{-1})

	x_1	x_2	x_3	x_4	x_5
x_1	0.495923	0.218481	−0.034481	−0.086644	−0.121665
x_2	0.218481	0.284289	−0.052504	−0.103538	−0.069718
x_3	−0.034481	−0.052504	0.023184	0.011691	0.019483
x_4	−0.086644	−0.103538	0.011691	0.099852	−0.015563
x_5	−0.121665	−0.069718	0.019483	−0.015563	0.561424

Two discriminant functions are used eventually, since they account for almost all the variation between varieties. The variation must be thought of as lying almost entirely in two dimensions corresponding to the two longest axes of the ellipsoid.

The two covariance matrices B and W are first calculated. This involves analysing the total covariance into its components as due to varieties (V), locations (L), years (Y) and their interactions, similar to the analysis of variance. The second order interaction V × L × Y was taken as the error or "within varieties" term. The average values of the 20-leaf samples were used as the basic figures in the analyses. Each character of each variety was represented by 15 basic figures (5 locations × 3 years).

Table 1 shows the S.P. for all sources of covariation and the correlation coefficients for "between varieties" and "within varieties". The S.S. for all sources of variation of the same data have been reported in the previous paper (7).

The S.S. and S.P. within varieties are given in a matrix form in Table 2, and designated as S.P. matrix within varieties or VLY matrix or W. The inverse matrix of VLY or W^{-1} was then calculated using the abbreviated Doolittle method, as presented by Goulden (6). The resulting inverse matrix of VLY is given in Table 3.

The S.S. and S.P. between varieties are similarly compiled in a matrix form and it is called S.P. matrix between varieties or B (Table 4). Both B and W are symmetrical matrices, that is $b_{ij} = b_{ji}$, $w_{ij} = w_{ji}$, respectively.

TABLE 4.—S.S. AND S.P. MATRIX BETWEEN VARIETIES (B)

	x_1	x_2	x_3	x_4	x_5
x_1	17.0474	−51.3943	−78.2319	−43.9613	−0.0389
x_2	−51.3943	328.1098	905.7257	207.1173	−46.8784
x_3	−78.2319	905.7257	3001.3982	478.9720	−189.7714
x_4	−43.9613	207.1173	478.9720	155.2574	−15.8742
x_5	−0.0389	−46.8784	−189.7714	−15.8742	14.8911

TABLE 5.—PRODUCT MATRIX OR BW^{-1} MATRIX

	x_1	x_2	x_3	x_4	x_5
x_1	3.736750	−2.224436	−0.217843	−1.459420	0.647175
x^2	2.725814	16.318864	7.051471	2.480661	−28.518043
x_3	37.184134	46.448938	26.630054	−1.129633	−109.147518
x_4	−4.586446	9.160191	3.251667	3.713946	−11.087869
x_5	−4.154145	−2.766349	−1.832467	0.821628	8.182956

Then the product matrix BW^{-1} or BW^{-1} matrix is calculated. This is done following the general rule of matrix multiplication. The resulting BW^{-1} matrix is presented in Table 5.

The values of λ, for which the determinant $|BW^{-1} - \lambda| = 0$, are found by expanding the determinant to calculate the polynomial

$$\lambda^5 - c_1\lambda^4 - c_2\lambda^3 - c_3\lambda^2 - c_4\lambda - c_5 = 0 .$$

The modified Frame method, as illustrated by Dwyer (3), was used to calculate the polynomial. The polynomial was found to be

$$\lambda^5 - 58.582570\lambda^4 + 574.652624\lambda^3 - 581.765879\lambda^2 + 131.376515\lambda - 0.019000 = 0 .$$

The roots of the above equation were calculated using the Graeffe root squaring technique described by Margenau and Murphy (8) and checked and corrected by using Horner's method of synthetic division.

The five roots were calculated to be

$$\lambda_1 = 46.489537$$
$$\lambda_2 = 10.982562$$
$$\lambda_3 = 0.781127$$
$$\lambda_4 = 0.329199$$
$$\lambda_5 = 0.000145 .$$

One can use the roots λ of the polynomial $|B - \lambda W| = 0$ to test the significance of the corresponding discriminant functions. The λ's represent the portions of the variation between varieties accounted for by the corresponding discriminant functions. One considers the statistic

$$\chi^2 = 25 \log_e (1 + \lambda) = 2.302585 \times 25 \log_{10} (1 + \lambda)$$

and compares it with tabulated chi-square with the appropriate number of degrees of freedom. Here $25 = (26 + 4) - \frac{1}{2}(5 + 5)$ = degrees of freedom due to "error" + d.f. due to varieties $- \frac{1}{2}$ (no. of characters + no. of varieties). The number of degrees of freedom for $25 \log_e (1 + \lambda_j)$, where λ_j is the j-th largest λ, equals (no. of characters + no. of varieties) $- 2j$.

Component of variation	Proportion of total variation	$25 \log_e (1 + \lambda)$	Degrees of freedom	Probability under null hypothesis
λ_1	0.7936	96.5129	8	<0.00001
λ_2	0.1875	62.0840	6	<0.00001
λ_3	0.0133	14.4309	4	0.0061
λ_4	0.0056	7.1144	2	0.0286
λ_5	0.0000	0.0036	0	—
All components	1.0000	180.1458	20	<0.00001

All but the last component are statistically significant. However, the first two components account for 98 per cent of the variation, and only two discriminant functions were computed. This enables one to make his decisions as to classification on the basis of a simple two-dimensional chart. An additional discriminant function might be useful in deciding borderline cases. The statistical significance of the third and fourth components may be due to the large number of observations upon which the calculations are based rather than to any large practical significance of these components. The number of components significantly different from zero cannot exceed the smaller of the two numbers, the number of characters and the number of degrees of freedom between varieties. Thus λ_5 is of no significance. The over-all test for all components confirms what we already knew from the analyses of variance of single characters, namely that the varieties are significantly different.

The two roots $\lambda_1 = 46.489537$ and $\lambda_2 = 10.982562$ are used in calculating the two characteristic vectors whose components are proportional to the coefficients of the first two discriminant functions. The Frame method, as presented by Dwyer (3), was used in calculations.

Now the two discriminant functions are constructed. Since only the ratios of the coefficients $k_1, k_2, \ldots, k_5$ matter, we could take one coefficient equal to unity and adjust the others proportionally. Instead, the

coefficients were so adjusted as to make the variance of the discriminant function equal to unity. The new discriminant function is called the standardized function and its coefficients are designated $K_1, K_2, \ldots, K_5$.

First discriminant function $z_1 = k_1x_1 + k_2x_2 + k_3x_3 + k_4x_4 + k_5x_5$,

where $k_1 = -75,229.54$ and x_1 = length of leaf blade
$k_2 = 836,520.03$ x_2 = width of leaf blade
$k_3 = 2,748,267.57$ x_3 = base angle
$k_4 = 441,286.51$ x_4 = apex angle
$k_5 = -174,255.38$ x_5 = length of petiole.

Its variance is

$$\begin{aligned}\mathrm{Var}(z_1) = {} & k_1^2\,\mathrm{Var}(x_1) + \ldots + k_5^2\,\mathrm{Var}(x_5) \\ & + 2\,k_1k_2\,\mathrm{Cov}(x_1, x_2) + \ldots + 2\,k_1k_5\,\mathrm{Cov}(x_1, x_5) \\ & + 2\,k_2k_3\,\mathrm{Cov}(x_2, x_3) + \ldots + 2\,k_4k_5\,\mathrm{Cov}(x_4, x_5)\,.\end{aligned}$$

$$K_1 = \frac{k_1}{\sqrt{\mathrm{Var}(z_1)}}, \ldots, K_5 = \frac{k_5}{\sqrt{\mathrm{Var}(z_1)}}$$

The new standardized function $z_1 = K_1x_1 + K_2x_2 + K_3x_3 + K_4x_4 + K_5x_5$; it has a variance of 1.

Likewise the second discriminant function $z_2 = l_1x_1 + l_2x_2 + l_3x_3 + l_4x_4 + l_5x_5$,

where $l_1 = -17,733.21$
$l_2 = 33,464.53$
$l_3 = -3,219.95$
$l_4 = 40,761.59$
$l_5 = 7,316.41$.

The new $z_2 = L_1x_1 + L_2x_2 + L_3x_3 + L_4x_4 + L_5x_5$ with a variance of 1.

Numerical values for the variances and coefficients of the first and second discriminant functions were then calculated and the coefficients of the standardized discriminant functions were found to be:

1st discriminant function	2nd discriminant function
$K_1 = -0.014166$	$L_1 = -0.356628$
$K_2 = 0.157524$	$L_2 = 0.672996$
$K_3 = 0.517521$	$L_3 = -0.064756$
$K_4 = 0.083098$	$L_4 = 0.819745$
$K_5 = -0.032814$	$L_5 = 0.147138$.

TABLE 6.—THE MEAN VALUES OF THE FIRST DISCRIMINANT FUNCTION FOR ALL VARIETIES

First discriminant function $z_1 = K_1x_1 + K_2x_2 + K_3x_3 + K_4x_4 + K_5x_5 =$

$= (-0.014166)x_1 + 0.157524x_2 + 0.517521x_3 + 0.083098x_4 + (-0.032814)x_5.$

$z_{\text{Elberta}} = K_1 17.2147 + K_2 28.3433 + K_3 92.90 + K_4 31.447 + K_5 4.4913 =$

$= -0.2439 + 4.4647 + 48.0777 + 2.6132 + (-0.1474) =$

$= 54.7643.$

$z_{\text{Vedette}} = K_1 16.5333 + K_2 27.7200 + K_3 90.19 + K_4 31.567 + K_5 4.5827 =$

$= -0.2342 + 4.5241 + 46.6752 + 2.6232 + (-0.1504) =$

$= 53.4379.$

$z_{\text{Veteran}} = K_1 17.8000 + K_2 26.8513 + K_3 91.05 + K_4 29.200 + K_5 4.1447 =$

$= -0.2522 + 4.2297 + 47.1203 + 2.4265 + (-0.1360) =$

$= 53.3883.$

$z_{\text{Spotlight}} = K_1 17.8320 + K_2 24.1093 + K_3 82.49 + K_4 28.027 + K_5 4.7247 =$

$= -0.2526 + 3.7978 + 42.6903 + 2.3290 + (-0.1550) =$

$= 48.4095.$

$z_{\text{Valiant}} = K_1 17.4887 + K_2 23.7020 + K_3 76.07 + K_4 28.793 + K_5 5.4920 =$

$= -0.2477 + 3.7336 + 39.3678 + 2.3926 + (-0.1802) =$

$= 45.0661.$

By substituting numerical values for K or L and x, the compound scores of the first and second discriminant functions are calculated. Table 6 shows the compound values of the first discriminant function for all varieties. A survey of the components of the compound value shows the amount to which each component contributes to the total compound value. In the first discriminant function, the magnitude of the total score was determined by the third component K_3x_3, which is the component corresponding to the base angle. Width of blade and apex angle made relatively small contributions while length of the blade and length of petiole were relatively unimportant.

In the second discriminant function (Table 7), the greatest contribution was made by the apex angle; width of blade was also an important component; length of leaf blade and size of base angle had moderate effect, but again length of petiole had small effect. The large effect of the apex angle could be partly attributed to the relatively high correlation between apex angle and width of leaf blade. It appears that length of petiole, although valuable in distinguishing Valiant from the other varieties, is generally of little value, and measurements of this character could be omitted.

TABLE 7.—THE MEAN VALUES OF THE SECOND DISCRIMINANT FUNCTION FOR ALL VARIETIES

Second discriminant function $z_2 = L_1x_1 + L_2x_2 + L_3x_3 + L_4x_4 + L_5x_5 =$
$= (-0.356628)x_1 + 0.672996x_2 + (-0.064756)x_3 + 0.819745x_4 + 0.147138x_5.$

$z_{\text{Elberta}} = L_1 17.2147 + L_2 28.3433 + L_3 92.90 + L_4 31.447 + L_5 4.4913 =$
$= -6.1392 + 19.0749 + (-6.0158) + 25.7785 + 0.6608 =$
$= 33.3592.$

$z_{\text{Vedette}} = L_1 16.5333 + L_2 28.7200 + L_3 90.19 + L_4 31.567 + L_5 4.5827 =$
$= -5.8962 + 19.3284 + (-5.8403) + 25.8769 + 0.6743 =$
$= 34.1431.$

$z_{\text{Veteran}} = L_1 17.8000 + L_2 26.8513 + L_3 91.05 + L_4 29.200 + L_5 4.1447 =$
$= -6.3480 + 18.0708 + (-5.8960) + 23.9366 + 0.6098 =$
$= 30.3732.$

$z_{\text{Spotlight}} = L_1 17.8320 + L_2 24.1093 + L_3 82.49 + L_4 28.027 + L_5 4.7247 =$
$= -6.3594 + 16.2255 + (-5.3417) + 22.9750 + 0.6952 =$
$= 28.2046.$

$z_{\text{Valiant}} = L_1 17.4887 + L_2 23.7020 + L_3 76.07 + L_4 28.793 + L_5 5.4920 =$
$= -6.2370 + 15.9514 + (-4.9260) + 23.6029 + 0.8081 =$
$= 29.1994.$

Since the variation is practically in two dimensions only, a chart can be simply constructed by plotting the compound scores for each variety mean on the two axes, z_1 and z_2. The chart so constructed is given in Figure 1. It well illustrates the grouping of the 5 varieties into 2 groups. Veteran, Elberta and Vedette formed one group which was characterized by high values on both axes, while the other group, represented by Valiant and Spotlight, was low particularly in the values on the first axis.

A simple technique was applied in dividing the chart into 5 regions corresponding to 5 varieties. After the average discriminant scores for each variety were calculated and plotted (indicated by ⊙) perpendicular bisectors were drawn to the lines joining the points ⊙. Every point on the graph is assigned to that variety whose average discriminant score it is closest to.

The values of the discriminant functions of any unknown sample can now be plotted on the chart to see where the point falls. As an example, all 15 samples of the Spotlight variety are plotted and shown in Figure 1 as small circles. The points are scattered rather widely and 5 samples are outside the variety region—they have been identified incorrectly. When all 75 samples were checked for correct identification, it was found that 31 samples or about 41 per cent were misclassified. The method giving so many chances for wrong identification, of course, could not be considered as satisfactory.

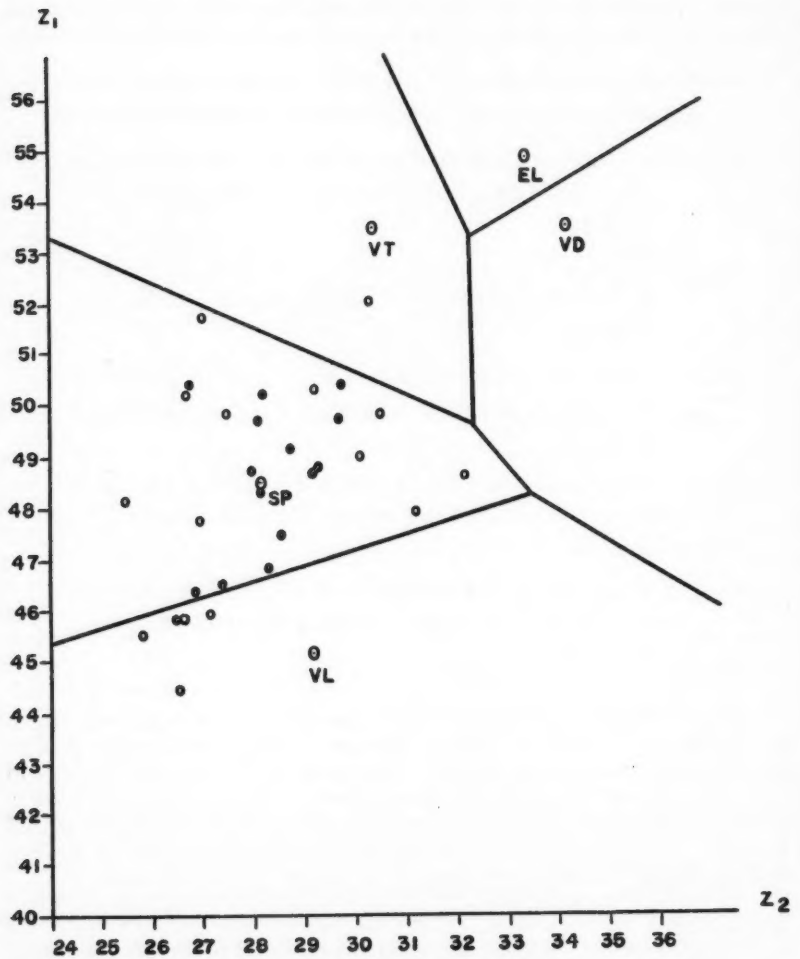

FIGURE 1. Positions of the values of varieties to two axes.

⊙ Mean values of Elberta (EL), Veteran (VT), Vedette (VD) Spotlight (SP) and Valiant (VL).

○ Individual samples of Spotlight, before adjustment.

● Individual samples of Spotlight, after adjustment for location effects.

The great deviation of the individual observations from the mean value was caused to a large extent by the location effect and by the interaction between locations and seasons. Investigation of deviations of the points of individual observations from the points representing variety general averages showed that usually at any one location in one season all deviations (for all varieties) were approximately of the same magnitude and

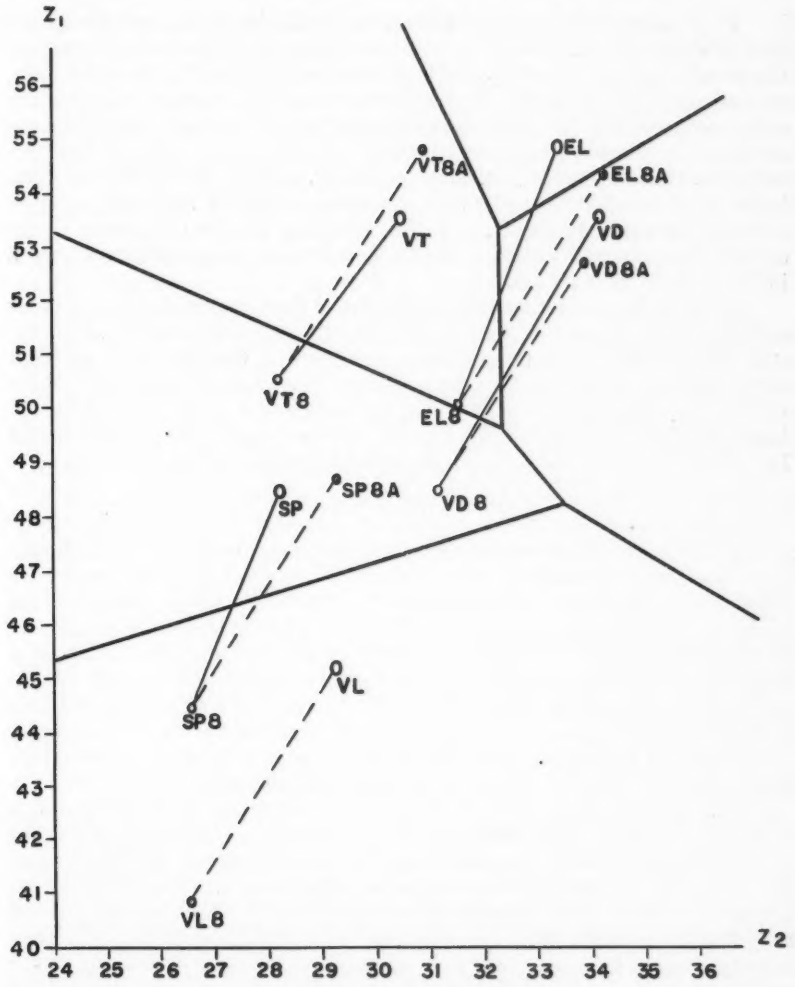

FIGURE 2. Deviation of sampling 8 from variety means.

- O Mean values of Elberta (EL), Veteran (VT), Vedette (VD), Spotlight (SP) and Valiant (VL).
  Positions of varieties in sampling 8, before adjustment.
- ● Positions of varieties in sampling 8, after adjustment for location effect.
- - - Adjustment line, —— deviation line. Narrow angles between the two lines in all varieties indicate similar trend in all varieties.

in the same direction. Consequently, if one variety, a standard, could be identified by visual observation and corrections were made for all varieties at the same location in the same amount as required for the standard, then chances of correct identification should be increased.

In Figure 2, the samples from location M in 1952 (samples 8) are plotted on the chart. In all varieties, deviation from the mean is towards the smaller values, especially on the z_1 axis, as indicated by the solid lines. If Valiant is taken as standard and the positions of other varieties are corrected according to standard, as indicated by interrupted lines, all points are brought close to variety averages, and out of four previously misclassified varieties, three are now identified correctly. Since the variety-location interactions were found to be non-significant a similar situation could be expected in other locations, and the adjusting of the compound scores could be considered justified. The adjusted values of Spotlight are shown in Figure 1 as filled circles.

When Valiant was taken as standard and the compound scores of other varieties were adjusted at each location to the same amount to which the standard deviated from the variety average score, then it was found that 12 samples or 16 per cent were misclassified. Considering that these results were obtained from 20-leaf samples and that a sample of 40 or 50 leaves would still be practical, a further increase in precision by the use of a larger sample could make the method useful for practical application.

DISCUSSION

Variation of the five measurements that were made on peach leaves was found to lie mostly in two dimensions and was expressed with two discriminant functions. The value of the two discriminant functions for each sample or variety average could be conveniently plotted as a point on the two axes, z_1 and z_2, of a chart. After each peach variety is assigned a region on the chart, identification of the unknown sample is very simple and consists of plotting the two compound values of the sample on the axes of the chart; the region where the point falls identifies the unknown sample.

When all 75 samples that were used in these studies were plotted on the chart, a relatively wide scatter of the samples was observed in each variety. The points of certain similar varieties were overlapping and a great proportion of the samples was misclassified. Analysis of variance of the data had shown that much of the scatter was caused by the effect of locations, seasons and their interactions. Of course, this kind of variation is non-existent if variety samples from one location in one season are treated separately. In doing so, it was observed that deviations of the individual variety observations from their over-all averages were approximately of the same magnitude. If deviation of the sample from the average was nullified for one variety and adjustment of the same magnitude was made for other varieties of that location, such a correction greatly reduced the scatter of the variety samples around their means. Misclassification of the samples with adjusted values was reduced to such a level that discriminant functions could find practical application. Most of the misclassified samples were found to lie close to the borderline of the variety region, and a larger, more representative sample would most likely bring them within the region of the correct variety. Adjustment of the values makes it necessary that one variety be positively identified without the use of discriminant functions and used as standard. Variety Valiant could successfully be used as standard. Certain leaf characters, such as long petiole, narrow base angle

of the leaf, many leaf glands, easily distinguished this variety from the other varieties under test. Valiant is a popular standard variety in British Columbia and is usually present in each nursery planting.

If corrections of the variety scores can be made for locations with extreme growing conditions, discriminant functions can be successfully applied in peach variety identification. Increase of the sample size would further improve the chances of correct classification. Since calculations are based on samples collected from 20 or more trees, it implies that groups of trees rather than individual plants are identified. Identification of individual trees by means of leaf measurements would be impracticable and in the majority of cases impossible.

Further work would be desirable in order to improve and simplify the method. First, the measurements of at least one character, viz. length of petiole, could be omitted. The possibility of omitting also the length of leaf blade could be considered. Of course, discriminant functions then should be reconstructed. Secondly, larger samples, e.g. consisting of 40 to 50 leaves, should be used. Increase of the sample size could be compensated by the decrease of the characters to be measured.

It appears that further work would make the use of discriminant functions of genuine value in identifying peach trees while still in the nursery, and the method should be practical for the trained man.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to C. A. Hornby, Associate Professor of Horticulture, University of British Columbia, for his great interest in the project and reviewing of the manuscript.

REFERENCES

1. Aitken, A. C. Determinants and matrices. 7th ed. Oliver and Boyd, Edinburgh. 1951.
2. Day, Besse B., and Marion M. Sandomire. Use of the discriminant function for more than two groups. J. Am. Statistics Assoc. 37 : 461-472. 1942.
3. Dwyer, P. S. Linear computations. John Wiley & Sons, New York. 1951.
4. Fisher, R. A. The use of multiple measurements in taxonomic problems. Ann. Eugenics 7 : 179-188. 1936.
5. Fisher, R. A. Statistical methods for research workers. 12th ed. Oliver & Boyd, Edinburgh. 1954.
6. Goulden, C. H. Methods of statistical analysis. 2nd ed. John Wiley & Sons, New York. 1952.
7. Lapins, K., and C. A. Hornby. Leaf measurements as a means of identification of peach varieties in nursery trees. Can. J. Agr. Sci. 36 : 409-421. 1956.
8. Margenau, H., and G. M. Murphy. The mathematics of physics and chemistry. D. van Nostrand Co., New York. 1943.
9. Panse, V. G. An application of the discriminant function for selection in poultry. J. Genet. 47 : 242-248. 1946.
10. Rao, C. R. Advanced statistical methods in biometric research. John Wiley & Sons, New York. 1952.
11. Rao, C. R., and P. Slater. Multivariate analysis applied to differences between neurotic groups. Brit. J. Psychology, Statistics Sec. 2 : 17-29. 1949.
12. Smith, H. F. A discriminant function for plant selection. Ann. Eugenics 7 : 240-250. 1936.

THE DISTRIBUTION AND IMPORTANCE OF THE CLOVER ROOT BORER (*HYLASTINUS OBSCURUS* (MARSH) COLEOPTERA: SCOLYTIDAE) IN QUEBEC[1, 2]

BURTON C. DEANE AND F. O. MORRISON[3]

[Received for publication April 3, 1956]

ABSTRACT

The clover root borer, *Hylastinus obscurus* (Marsh), occurred throughout western, southern and central Quebec in 1954. The roots of from 28 per cent to 94 per cent of the plants in some red clover stands became infested during the spring and summer of the second and third years of growth. Infestation intensity was not correlated with soil pH or texture.

Borer adults spread from the point of origin during a brief flight in the spring.

A cantharid larva, an adult staphylinid (*Grohypnus* sp.) and unidentified dipterous larvae were observed feeding on borer larvae.

Lindane applied in the spring increased clover hay yields on artificially infested plots.

The larger plants were more commonly infested and also more commonly infected with root rots. Laboratory experiments indicated that activity of adult borers could help to initiate fungal infections.

Very few plants which survived a second winter showed infestation in the following spring.

INTRODUCTION

Several factors appear to prevent red clover (*Trifolium pratense* L.) from attaining its potential productivity under Quebec conditions. One of these may be the clover root borer [*Hylastinus obscurus* (Marsh.)], which has for many years been noted in a large percentage of clover roots in Eastern Canada and the United States (5, 6, 8, 9). The present study was undertaken to determine the biology of this species in Quebec and to assess its importance.

MATERIALS, METHODS AND PROCEDURE USED

In Studying Distribution

Root samples were taken from first- and second-crop-year red clover fields at approximately 2-mile intervals along main and side roads throughout the main agricultural areas of the Province of Quebec during the summers of 1953 and 1954. A root sample consisted of 15 plants selected at random over the field and dug up with a spade. Though usually only one sample was taken from a field, some large fields and certain fields under intensive study were sampled several times. No attempt was made to select individual plants on either a size or condition basis. The plant bundles were labelled and stored at 38° F. until the roots could be carefully dissected under a binocular microscope. The crown diameter, presence or absence of root rots, and the number of borers in each developmental stage were recorded. In 1954, a cylindrical one-quart cardboard carton was filled in the field with soil from about the roots of each plant sample dug up. The pH of this soil was determined later using a Beckman model G meter.

[1] Part of thesis submitted to McGill University by the senior author in October 1954 in partial fulfilment of the requirements for the degree of M.Sc.Agr. *Macdonald College Journal* Series No. 385.

[2] This investigation was supported by a grant-in-aid from the Quebec Agricultural Research Council.

[3] Associate Professor of Entomology, Macdonald College, McGill University.

In Life History and Habit Studies

The seasonal history and habits of *Hylastinus obscurus* were investigated, using the stages found in the dated field survey samples, in similar samples of 30 plants each taken weekly during 1953 from first- and second-crop-year fields at Macdonald College, and by trapping adults on masonite boards 2 feet square covered with Tanglefoot and erected on short stakes in clover fields. Eighty such traps were examined daily at 8.00 a.m. Eastern Standard Time from April 28 to May 19, 1954. On several occasions examinations were made after sundown and again at the usual time in the morning. Continuous records of air temperature 4 inches above the soil were taken with field thermographs under small open shelters in the fields being studied. During the period of flight, cloud type and degree of cloudiness were recorded every 2 hours according to a scale suggested by Geiger (1) while wind velocity was estimated according to the Beauford Scale.

In Studying the Interrelations of Borers and Clover Diseases

A group of 20 adult borers dissected from roots collected in the field was allowed to move about for one hour on Petri dish cultures of the causal organism of *Sclerotinia* crown and stem rot (*Sclerotinium trifoliorum* Erik.)* Another group was similarly exposed to cultures of the northern anthracnose organism (*Kabatiella caulivora* (Kirchn) Karak)*. Individual borers were removed and caged on potted Dollard clover (clonal stock raised from cuttings). The borers were held on the crowns of the plants by enclosing them in small cages made by placing the smaller end of a gelatin capsule inside the larger and burning air holes through the double wall of the closed end with a hot needle. The cage with the borer inside was then attached with paraffin wax to the crown of a plant. The plant were held in a humidity chamber at 75 per cent relative humidity and later examined for disease symptoms. Tissue from plants showing symptoms was cultured on potato dextrose agar to verify infection.

Another test was carried out with two lots of 8 beetles exposed for one hour on *S. trifoliorum* and two more on *K. caulivora* cultures. One lot of beetles from each culture was surface disinfected with 1:1000 mercuric chloride in water for 3 minutes. Beetles from both lots from each culture were macerated individually and placed on sterile potato-dextrose agar plates.

In Studying Losses Caused by the Borer

Forage yields were taken on plots from which beetles had been excluded, on plots to which chemical controls had been applied, and on artificially infested plots with and without chemical treatment. This experiment was conducted in the spring of 1954 on a pure clover stand entering its first-crop-year and showing no infestation at the beginning of the tests. The plots were 4 feet by 4 feet and separated by 1 foot-wide pathways. The design was a 5 by 5 Latin square. Where it was desired to prevent infestation, the plots were covered with a closely woven cotton cloth, stretched on a frame 10 inches from the ground, and fastened tightly and securely to the ground around the edges by means of wooden strips

*Courtesy W. Kreitlow, Bureau of Plant Industry, Beltsville, Maryland, U.S.A.

fastened to the edge of the cloth and pegged down with notched stakes. Beetles dissected from plants collected elsewhere were added to certain plots at the rate of two per plant. This was done some 3 weeks after the field flight as no beetles could be secured prior to that time. Lindane in the form of a 25 per cent wettable powder was applied at 1½ lb. of gamma isomer per acre to uncovered plots on May 1 and on May 22 (two days before beetles were added) to plots that were to be artificially infested. The insecticide was applied in water using a 2-gallon garden watering can. All covered plots were uncovered on July 17. The plants from an area of one four-thousandth of an acre on each plot were harvested on August 5 and the green material immediately weighed. Observations were made on the infestation of living plants in the same field in the fall and following spring to assess the borer as a factor in winter plant mortality.

RESULTS

Distribution

The survey conducted in 1953-1954 established that *Hylastinus obscurus* was present in most clover-growing areas of the Province (Table I). Soil samples from the 35 fields in 13 counties sampled in 1954 varied in pH from 5.4 to 7.7 and in texture from gravelly loam through fine sandy loam to heavy clay but no correlation could be found between either the soil pH or its physical texture and the intensity of infestation.

Life History, Habits and Natural Control Factors

Eggs were found from May 1 on. The first larvae occurred in roots taken on June 16, 1953. On July 7, ten larvae were found in one plant. The stages overlapped but the peak of larval occurrence was from July 27 to July 30; that of pupal occurrence from August 29 to September 5. Adults were infrequent in collections between July 30 and August 5.

TABLE 1.—PERCENTAGE OF SECOND-CROP-YEAR RED CLOVER ROOTS FOUND INFESTED WITH CLOVER ROOT BORER, ARRANGED BY COUNTIES (1953-54).

County	Percentage infestations	Number of samples[1]
Argenteuil	0	2
Beauharnois	22	8
Brome-Missisquoi	15	7
Chambly	15	9
Chateauguay	31	24
Hull	13	5
Huntingdon	4	23
Jacques Cartier	36	300[2]
Labelle	11	4
Nicolet-Yamaska	23	2
Normandin	0	1
Sherbrooke	2	4
St. Jean	11	6
Stanstead	0	3
Vaudreuil	11	12

[1] As a rule, one sample was taken per field.
[2] A high percentage of these samples came from a few fields.

Very few beetles were found in living plants collected in the spring prior to the adult flight. A sample of 75 plants taken in the early spring in 1954 from a field, which in August 1953 (its first crop-year) yielded samples with 20 per cent of the roots infested, showed only 1 per cent infestation. Plants known to be entering their third winter were invariably infested when examined in the fall.

Two fields, a first- and a second-crop-year field, were examined weekly from May 15 to September 11, 1954. During April and May there were no important differences in infestation between the two fields from 7 to 10 per cent of the examined roots being infested. Table 2 shows that differences increased as the summer progressed.

A total of 39 beetles were caught by the 80 sticky trap-boards exposed from April 28 to June 19. The first 4 beetles were caught on a board in the second-crop-year field on May 2. They were found when the boards were examined on the morning of May 3. Since May 3 was wet no beetles were found when the boards were examined on May 4. Twenty beetles were taken from the boards in the second-crop-year field and 5 in the adjacent first-crop-year field on May 5. This was the highest catch for any one day. Seven were taken on May 6, 4 on May 7, 1 on May 10, 2 on May 15 and 1 on May 17. The direction of flight to the growing stands was from a field which, after having been cropped for two successive years, had been ploughed down the previous September. The crop had been 80 per cent infested in the late summer of 1953. The maximum air temperature recorded during the first half of May was 78°F. [cf. Gyrisco (3)].

On four occasions all traps were examined after sunset and again in the morning. No flights occurred during the hours of darkness nor on days when there was continuous drizzle or appreciable cloud cover. Winds did not exceed 12 miles an hour during the flight period and did not appear to influence the activity or direction of flight.

TABLE 2.—PER CENT BORER-INFESTED ROOTS IN ADJACENT SECOND-CROP-YEAR AND THIRD-CROP-YEAR RED CLOVER FIELDS, QUEBEC, 1954

Weed of	Infestation percentages	
	First crop year	Second crop year
April 30	0	0
May 15	6.7	10.0
June 7	13.3	28.6
June 14	13.3	28.6
June 28	13.3	50.5
July 4	28.6	83.3
July 12	28.6	87.0
August 5	—	94.0
August 12	28.6	94.0
September 7	—	94.0

On one occasion a cantharid larva and on another an adult staphylinid (*Grohypnus* sp. very near *humatus* Say*) were observed feeding on root borer larvae in the field. Unidentified dipterous maggots were noted feeding externally on borer larvae on several occasions.

Interrelations of Borer and Clover Diseases

Table 3 shows the association of crown diameter with infestation.

TABLE 3.—ASSOCIATION BETWEEN CROWN DIAMETER AND BORER INFESTATION

Crown diameter in mm.	Infested		Non-infested		Total plants measured
	Obs.	Exp.	Obs.	Exp.	
2-7	27	50.6	215	191.4	242
8-12	85	82.2	308	310.6	393
13-18	30	13.2	33	49.8	63
19-22	5	1.0	0	4.0	5
	147		556		703

$$\text{Chi Square} = \frac{(\text{observed} - \text{expected})^2}{\text{expected}} = 53.37 \text{ for 3 degrees of freedom}$$

If infestation were random, i.e., unrelated to root diameter, Chi^2 should be zero. At a probability ($p = 0.01$) that distribution is not random Chi^2 would be 11.341.

There was also a marked association between crown diameter and the presence of root rots (Table 4).

Though an exact count was not kept, it can be said that infested roots free of rot were rare but rot did occur in some apparently uninfested roots.

TABLE 4.—ASSOCIATION BETWEEN CROWN DIAMETER AND ROOT ROT

Crown diameter in mm.	Root rot		No root rot		Total plants measured
	Obs.	Exp.	Obs.	Exp.	
2-7	146	164.6	87	68.4	233
8-12	285	254.3	95	105.8	380
13-18	50	41.7	9	17.3	59
19-22	3	3.5	2	1.5	5
	484		193		677

$$\text{Chi Square} = \frac{(\text{observed} - \text{expected})^2}{\text{expected}} = 14.44 \text{ for 3 degrees of freedom}$$

With a Chi^2 of 11.341 the probability of random distribution of infection is $P = 0.01$.

* Determination by W. J. Brown, Entomology Division, Science Service, Ottawa.

TABLE 5.—WEIGHT OF GREEN PLANTS HARVESTED ON AUGUST 5, 1954, FROM RED CLOVER PLOTS DESIGNED TO INVESTIGATE LOSSES DUE TO THE CLOVER ROOT BORER

Treatments	Mean of 5 plots in grams
A. Uncaged, naturally infested, sprayed	2,484
B. Uncaged, naturally infested	2,135
C. Caged, artificially infested, sprayed	1,599
D. Caged, artificially infested	1,148
E. Caged before beetle flight	1,308
L.S.D. at P = 0.05 = 391.7	

Fourteen of 20 potted clover plants, each of which had been artificially infested with a single borer adult previously exposed in an *S. trifoliorum* culture, developed disease symptoms within seven days. Six of 20 plants similarly infested with adults previously exposed to *K. caulivora* likewise exhibited a diseased condition. Fungal organisms were cultured from plants showing symptoms.

Six of 8 potato-dextrose-agar plates each inoculated with one macerated borer adult previously exposed on a culture of *S. trifoliorum* and surface disinfected before maceration exhibited fungal growth. Beetles exposed to *K. caulivora* and treated as above caused growth on 4 out of 8 plates. Non-surface disinfected adults similarly exposed and macerated produced fungal growth on all plates. In all instances, the authors thought the symptoms and growth on agar to be typical of the organisms being worked with, but were not in a position to identify them positively.

Field Losses due to the Borer.

Only 8 borer infested roots were found among 720 living roots examined in the spring from a field in which 20 per cent of the roots had been infested the previous fall. This suggests a high mortality of infested roots during the third winter of the clover plant's life.

The mean weights of the green plants grown on treated, covered and artificially infested plots in 1954 are given in Table 5 and the analysis of variance on these weights in Table 6.

DISCUSSION AND CONCLUSIONS

The presence of borer is easily determined from the dissection of 15-root samples, but the comparative intensity of infestation is only measurable if the samples are taken at approximately the same time of year. Thus Table 1 indicates the occurrence of the borer throughout 2 years, but not the relative intensity of infestation. The highest intensity for the year is not attained until August (Table 2). The small size of the eggs and newly hatched larvae, and the winter killing of infested roots make samples taken before July poor indicators of infestation.

TABLE 6.—ANALYSIS OF VARIANCE OF GREEN PLANT WEIGHTS

Source	df	DD	MS	F	0.05	0.01
Row	4	383,889	95,972	1.2		
Column	4	565,576	141,394	1.7		
Treatment	4	6,568,770	1,642,192	20.3	3.26[1]	5.41[2]
A vs B[3]	1	304,154		3.8	4.75	9.33
C vs D[3]	1	508,502		6.2	4.75[1]	9.33
AB vs CD[3]	1	4,399,544		54.2	4.75[1]	9.33[2]
E[3] vs all others	1	1,376,570		17.0	4.75[1]	9.33[2]
Error	12	970,525	80,877			
TOTAL	24	8,488,760				

[1] Differences significant at P = 0.05.
[2] Differences significant at P = 0.01.
[3] Letters refer to treatments as in Table 5.

The seasonal history of *H. obscurus* in Quebec as inferred from stages found on different dates is as follows:

Eggs are laid from May 1 to July 25
Larvae occur from June 15 to September 15
Pupae occur from July 25 to November 5
Adults occur from August 10 to July 10 of the following year. (cf. 5, 6, 8 and 9).

The dispersal flight occurs only once each year. Gyrisco (2) found it to be "triggered" by the occurrence of two successive days with maximum temperatures of 65°F. and to be independent of maximum temperature thereafter. The observed flight at Macdonald College appeared to bear out Gyrisco's conclusions. The major part of the flight occurred on one day.

Predation and parasitism of larvae were occasionally observed but they are unlikely to be important factors in the control of this insect.

A significant association between plant crown diameter and infestation suggests that the adult borers select the larger plants in which to oviposit. A similar association between plant diameter and root rots indicates that a high per cent of the infested roots were also rotted. How the percentages of rotted roots compared when infested and non-infested roots of the same size range are compared is something which should be determined.

The presence of a much smaller percentage of infested roots in samples of living plants taken in the spring of the second-crop-year than was evident in plants from the same field the previous fall indicates a higher winter mortality among non-infested plants. Since infested and non-infested plants cannot be determined without disturbing the plant, and since by the time growth has commenced in the spring, the roots of some winter-killed plants are rotted almost beyond recognition, direct comparisons of mortality in infested and uninfested plants could not be made. It is hoped to do this in the future by artificially infesting marked plants.

The laboratory evidence that borer adults can carry the fungal organisms of known diseases suggests that losses from this pest may result from its activities as a vector of disease as well as from direct feeding injury. *Kabatiella caulivora* sporulates readily in culture and could have been transferred in this form. *S. trifoliorum* does not form spores in culture*. It must have been carried as hyphal pieces or else the infection was due to other fungi in the culture, on the plant or on the beetles when they were collected. In either case the activity of the insects accounted for the infections. These results are in keeping with those reported by Miller (4). Since the beetles that fly in the spring emerge from winter-killed roots, a high percentage of which were diseased, the opportunity for disease spread is great.

Definite proof of serious losses due to the clover root borer in the east has proved difficult to secure, though Rockwood (5) reported instances of spectacular damage to both first and second-crop-year clover in Washington State and refers to such damage having been observed in several states including New York. Gyrisco and Marshall (2) found no significant increase in hay yield when chemical control was effected, but Weaver and Haynes (7) have since this work was completed produced evidence for increased hay yields accompanying chemical control in Ohio. The necessity of using a very heavy cotton to retain the small beetles or covering field plots in our experiments and the further necessity of keeping the plots covered until adequate supplies of beetles for artificial infestation could be secured, so reduced the growth in the covered plots, due to interference with light, that the only valid comparison is between yields from caged and artificially infested plots which were sprayed and similar plots unsprayed. Spraying appears to have offered some protection from damage by heavy artificial infestations.

REFERENCES

1. Geiger, R. The climate near the ground (Trans. by M. N. Stewart *et al.*). Harvard Univ. Press, Cambridge, Mass. 1950.
2. Gyrisco, G. G., and D. S. Marshall. Further investigation on the control of the clover root borer in New York. J. Econ. Entomol. 43 : 82-86. 1951.
3. Gyrisco, G. G., A. A. Muka, L. Hopkins, and H. H. Neunzig. Insecticide concentrations and timing of applications for the control of the clover root borer. J. Econ. Entomol. 47 : 327-331. 1954.
4. Miller, P. R. Plant disease situation in the United States. FAO Plant Prot. Bull. 3 (10) : 148-151. 1955. *Cited in:* Rev. Appl. Entomol. Ser. A. 44 : 114-115. 1956.
5. Rockwood, L. P. The clover root borer. U.S.D.A. Bull. 1426 : 1-48. 1926.
6. Riley, C. V. The clover root borer. U.S.D.A. Annual Report 1878 : 248-250. 1879.
7. Weaver, C. R., and J. L. Haynes. Band placement of insecticides for clover root borer control. J. Econ. Entomol. 48 : 190-191. 1955.
8. Webster, F. M. The clover root borer. Ohio Agr. Expt. Sta. Bull. 112 : 143-149. 1899.
9. White, J. The clover root borer, *Hylastis trifolii*, Muller. Can. Entomologist 20 : 138. 1888.

* Personal communication from R. Estey, Plant Pathology Department, Macdonald College, Que.

A COMPARISON OF THE LINE INTERCEPTION, VERTICAL POINT QUADRAT, AND LOOP METHODS AS USED IN MEASURING BASAL AREA OF GRASSLAND VEGETATION[1]

A. JOHNSTON[2]

[Received for publication May 24, 1956]

ABSTRACT

A study was designed to determine the most efficient sample size and number of samples when any one of the line-interception, vertical point quadrat, or loop methods of vegetation survey is used, on different range types in southern Alberta. The procedure has involved obtaining basal area measurements by all methods on ten transects, 100 feet long, within each range type.

Sampling intensities sufficient to sample the dominant and minor species to within ± 10 per cent of their true mean basal area, and the precision obtained when minor species were sampled at intensities found adequate for the dominant species, were calculated. Measurement of minor species at the latter intensities was highly variable. The loop method was the most rapid in the field, detected least species, and gave the most variable data. Data collected by this method were especially variable when the dominant plant species were of the single stemmed type. The line interception method was the most time-consuming, detected more species than either the loop or point methods and gave data which were slightly more variable than the point method. The point method gave the least variable data and, in general, seemed the most satisfactory of the three methods in the vegetative types studied.

INTRODUCTION

Of the many methods of vegetation analyses available to the range technician or ecologist, three are of particular interest. These are: *the line interception* (1), *the vertical point quadrat method* (12), and *the loop method* (14). The line interception method, in addition to its development by Canfield (1), has been used by Savage (15) in dune vegetation of the Southern Great Plains, by Canfield (2) in measuring utilization of grasslands, and by Hormay (11) in measuring density and yield on bunch grass types in California. Hanson (10), in his discussion of vegetation survey methods, concluded that the line interception method was most suited to a mixed grass-forb-shrub vegetation and was not as satisfactory as other methods in dense grassland types.

The vertical point quadrat method has been used extensively throughout the world (8). Crocker and Tiver (6) in Australia, and Clarke and Campbell (3) and Coupland (4) in Canada, have used the method to determine density and composition of grassland types. In the United States Hanson (9) was one of the first to use the method and it has been tested by Tinney *et al.* (18) and Ellison (7) among others. Whitman and Siggeirsson (20) have compared the line interception and point quadrat methods as used in analysing grass vegetation in North Dakota.

[1] Contribution from the Forage Crops Division, Experimental Farms Service, Canada Department of Agriculture. (This paper is based partly on a thesis presented by the author to the Graduate School, Montana State College, in partial fulfilment of the requirements for the degree of Master of Science in Range Management).

[2] Experimental Farm, Lethbridge, Alberta.

The loop method (14) was developed for use in appraising condition and trend in national forest ranges in the United States. Literature to date has dealt primarily with improvements in technique (17) or tests of its adaptability (16).

The objectives of the study being reported were to compare these three methods as to the number and size of samples needed to secure a reliable estimate of basal area, and to determine to what extent measurements obtained by these methods were influenced by the methods themselves.

METHODS

Four sites in southern Alberta were selected for the study. These were located at Stavely, representing the *Festuca-Danthonia* faciation of the fescue grassland; at Coalhurst, representing the *Agropyron-Stipa* associes of the mixed prairie (13); and at Lethbridge and Vauxhall, representing the *Bouteloua-Stipa* faciation of the mixed prairie (4).

At each site a uniform area was selected by visual inspection. A total of ten transects, each 100 feet long, placed 10 feet apart, and parallel to each other, were then laid out at each site. A metal tape, 100 feet long, graduated in 1/100th's of a foot, was stretched tightly along each transect in turn as close to the ground surface as possible and anchored at each end by a steel pin. Measurements by each method were made along the edge of this tape.

When measuring by the line interception method, the interception of plant crowns, on a vertical plane directly below the edge of the tape was measured to the nearest 1/100th foot and recorded by species. Measurements by the point method were made by placing a frame containing 36 pins, spaced one inch apart, directly over the tape in such a way that the pins touched the edge of the tape along its length. Points were, therefore, examined at one-inch intervals along the 100-foot length of the tape and crown hits of species were recorded. When using the loop method, a $\frac{3}{4}$-inch loop was dropped at intervals of 1 foot along the length of the tape in such a way that a vertical plane below the edge of the tape would bisect the loop. Crown portions of vegetation appearing in the loop were recorded by species. Data were recorded in such a way that each 10-foot length of each 100-foot transect could be later identified. Therefore, at each site, the per cent of species present, obtained by three methods on 100 plots, 10 feet long, were available for study and analysis.

Measurements were taken during August, 1952, when the grasses were nearly mature. All areas had been protected from use by livestock for several years prior to the study.

One of the main purposes in the statistical analysis of the data was to determine sampling intensities that would give a desired level of accuracy. To this end, a procedure utilized by Torrie *et al.* (19) was followed.

Torrie *et al.* (19) pointed out that field experiments in which samples are taken to represent the whole plot have two sources of random variation These are the sampling error and experimental error. In the study being reported, the sampling error is represented by the variance between plots within transects. Experimental error is made up of the sampling error plus the random variation among transects. These two sources of vari-

ation are independent of each other. Thus, the random variation among transects is designated as "A" and the variance of the mean of "n" plots about the mean of the transect as $\frac{B}{n}$. The experimental error variance in terms of transect means would be $A + \frac{B}{n}$ which, in terms of the individual samples, would be $n\left(A+\frac{B}{n}\right)$ or $nA + B$. The estimated variance of a species mean on a single plot basis, V_x, would be $V_x = \frac{nA + B}{nk}$ where "n" is the number of 10-foot plots, and "k" is the number of transects.

By a variance analysis of the data obtained by one method at one site and knowing the values of "n" and "k" used in the variance analysis, A and B can be calculated. By substituting a value of V_x equal to 10 per cent of the mean basal area squared, the sampling intensity (i.e. the values of "n" and "k") required to attain this level of accuracy can be calculated.

These calculations are illustrated, using data obtained by the line interception method at the Stavely site. The basal area of the dominant grass species (*Danthonia parryi* Scribn.) has been used in the calculations.

Experimental error	(nA + B)	= 63.35
Sampling error	(B)	= 25.54
	nA	= 37.81
	A	= 3.78

To sample within ± 10 per cent of mean, V_x must equal 0.883.

$$\text{Therefore, } 0.883 = \frac{n(3.78) + 25.54}{nk}$$

and n = 5, and k = 10

Therefore, 10 transects, each 50 feet long, will sample vegetation to within ± 10 per cent of mean basal area of the dominant grass species. (*See Table 4*).

RESULTS AND DISCUSSION

Differences existed in the estimate of basal area obtained by each of the three methods as is shown by the data in Table 1. One of the more surprising differences was the consistently higher values obtained by the point quadrat method as compared with the line interception method. In theory, since a point has position but no area, these results should not have been obtained. It is felt that pin diameter may have caused this difference since the pins used were 3/32 inch in diameter and had a rather blunt point. In practice it appears that a tiny loop was being recorded each time, rather than a point. The differences between methods were much greater at the Coalhurst site in the case of *Agropyron Smithii* Rydb., and can be attributed to the scattered growth habit and numerous individual culms of this species. Estimates by the loop method were extremely high for this grass—nearly five times that obtained by the line interception method. In this connection a proposal has been made (5) that plants appearing in the loop be recorded, or in those cases where no plant appears,

TABLE 1.—AVERAGE PER CENT BASAL AREA OF VEGETATION ON FOUR STUDY AREAS AS DETERMINED BY THE LINE INTERCEPTION, POINT QUADRAT, AND LOOP METHODS

	Line	Point	Loop
Stavely			
Danthonia parryi Scribn.	10.89	12.00	24.55
Festuca scabrella Torr.	3.44	3.87	8.40
Agropyron subsecundum (Link) Hitch.	0.19	0.22	1.30
Stipa spartea Trin. var *curtiseta* Hitch.	0.10	0.14	0.80
Koeleria cristata (L.) Pers.	0.04	0.11	0.45
Other grasses	0.43	0.55	2.06
Carex spp.	0.58	1.15	7.60
Forbs	0.59	0.66	1.74
Total	16.26	18.70	46.90
Coalhurst			
	Line	Point	Loop
Agropyron smithii Rydb.	2.99	5.32	14.65
Stipa viridula Trin.	2.03	2.52	3.95
Stipa comata Trin. and Rupr.	0.81	1.22	2.90
Bouteloua gracilis (H. B. K.) Lag.	0.62	0.70	1.40
Koeleria cristata (L.) Pers.	0.45	0.64	1.30
Carex spp.	2.29	3.65	18.35
Forbs	0.70	1.05	2.85
Total	9.89	15.10	45.40
Lethbridge			
	Line	Point	Loop
Bouteloua gracilis (H. B. K.) Lag.	31.67	33.83	64.30
Stipa comata Trim. and Rupr.	1.86	1.90	3.70
Poa secunda Presl.	1.31	1.25	3.55
Agropyron smithii Rydb.	0.28	0.25	1.20
Koeleria cristata (L.) Pers.	0.03	0.13	0.00
Carex spp.	0.73	0.79	3.15
Forbs	0.71	1.08	1.30
Total	36.59	39.23	77.20
Vauxhall			
	Line	Point	Loop
Bouteloua gracilis (H. B. K.) Lag.	19.95	20.62	40.25
Stipa comata Trin. and Rupr.	2.50	2.31	4.04
Poa secunda Presl.	1.00	1.09	4.00
Koeleria cristata (L.) Pers.	0.77	0.79	2.20
Agropyron smithii Rydb.	0.64	0.69	2.40
Other grasses	0.09	0.07	1.00
Carex spp.	0.39	0.27	2.80
Forbs	18.80	22.44	25.05
Total	44.14	48.28	81.74

those nearest the loop be recorded. The procedure has been called the "nearest hit technique". In view of the high values obtained when measuring basal area of *Agropyron Smithii* Rydb. the procedure should be used with caution.

Table 2 shows the number of species encountered by each method at each site. The three methods detected approximately the same number

TABLE 2.—NUMBER OF SPECIES ENCOUNTERED WHEN MEASURING DENSITY OF VEGETATION BY THREE METHODS AT FOUR SITES

Vegetative Class and Site	Method		
	Line interception	Point	Loop
Grasses and Carices			
Stavely	11	11	10
Coalhurst	6	6	6
Lethbridge	7	6	5
Vauxhall	7	7	7
Average	7.75	7.50	7.00
Forbs and Shrubs			
Stavely	24	20	11
Coalhurst	7	6	7
Lethbridge	6	9	5
Vauxhall	11	8	6
Average	12.00	10.75	7.25

TABLE 3.—ESTIMATED TIME IN MAN-HOURS REQUIRED TO MEASURE AND RECORD DENSITY OF VEGETATION PER 100-FOOT TRANSECT BY THREE METHODS AT FOUR SITES

Method	Stavely	Coalhurst	Lethbridge	Vauxhall
Line interception	2.50	1.75	2.00	2.00
Point quadrat	0.75	0.50	0.65	0.65
Loop method	0.40	0.25	0.30	0.30

TABLE 4.—SAMPLING INTENSITIES REQUIRED TO SAMPLE THE DOMINANT SPECIES TO WITHIN ± 10 PER CENT OF THEIR RESPECTIVE MEANS USING BASAL AREAS OBTAINED BY THREE METHODS AT FOUR SITES

Site	Line Interception Method		Point Method	Loop Method		
	Transects	Length	Points	No. transects	Length	No. transects/ cluster
Stavely	10	50′	2400	25	100	2
Coalhurst	10	50′	3000	68	50	7
Lethbridge	6	50′	1200	2	100	1
Vauxhall	15	50′	4320	11	100	2

of grass species but differed in the number of forb and shrub species encountered. The reason for this difference appeared to be related to the infrequent occurrence of forb and shrub species throughout the study sites. While they might be encountered when using the line interception method, they were very easily missed when using either the point or loop methods.

The estimated time spent in obtaining and recording data by each method at each site is shown in Table 3. The data show that with increasing density and complexity of the vegetative cover the time required when using the line interception method increased considerably. This is not so when using the point and loop methods since, regardless of the cover, each point or loop has to be examined and, if the observer is familiar with the species growing in an area, the necessary information can be obtained rapidly. The slight increase shown for these two methods at the Stavely site is due largely to the increased number of species which had to be written into the field record. The amount of litter on the ground surface was a problem with all methods, particularly at the Stavely site. The presence of large amounts of this material was a factor in the time spent at that site.

Sampling intensities were calculated using two groups of grass species. These were the dominant grass species at each site, and a group of secondary species, whose cumulative basal area was in the order of 1 per cent at each site. Intensities of sampling required to sample the dominant species to within $\pm$ 10 per cent of their respective true means are shown in Table 4. In this table it will be noted, under the line interception method, that transects 50 feet long are suggested as being satisfactory at each site. This length was selected somewhat arbitrarily as the data showed a shorter transect to be much more efficient per unit of length. For instance, at the Stavely site a total of 33 transects, each 10 feet long, for a linear sampling area of 330 feet, would sample the dominant species to the level of accuracy desired. The same accuracy could be obtained using 7 transects, each 100 feet long, for a linear sampling area of 700 feet. However, since the work involved in relocating short transects many times is appreciable it is felt that work and time would be saved by utilizing fewer transects of a longer length.

In the case of the loop method a procedure outlined by Parker (14) was used in calculating the most desirable number of loops per transect. Since the loops were dropped at intervals of 1 foot, these figures also gave the most desirable transect length. At all sites except Vauxhall, transects 100 feet long seemed most suitable while at Vauxhall a transect 50 feet long proved more satisfactory.

Parker (14) suggests setting up transects, when using the loop method, in clusters rather than scattering them at random over the field. He also gives a method for calculating the most desirable number per cluster. Following his procedure the numbers of transects per cluster at each site were calculated and are shown in Table 4.

In practical sampling minor constituents of the sward may be of real importance. For this reason, sampling intensities were calculated at each site for a group of minor species whose cumulative basal area was in

TABLE 5.—SAMPLING INTENSITIES REQUIRED TO SAMPLE SECONDARY SPECIES TO WITHIN ± 10 PER CENT OF THEIR RESPECTIVE MEANS USING BASAL AREAS OBTAINED BY TWO METHODS AT FOUR SITES

Site	Line Interception		Point Method
	No. transects	Length	No. of points
Stavely	27	50	36600
Coalhurst	95	50	38940
Lethbridge	46	50	22560
Vauxhall	42	50	23280

TABLE 6.—SAMPLING PRECISION OBTAINED WHEN SAMPLING SECONDARY SPECIES AT INTENSITIES FOUND ADEQUATE FOR THE DOMINANT SPECIES AT EACH SITE BY TWO METHODS

Site	Line Interception Method		Point Method	
	Chances 19:1 that the sample mean will be within the following per cent of the true mean		Chances 19:1 that the sample mean will be within the following per cent of the true mean	
	Dominant	Secondary	Dominant	Secondary
Stavely	± 10%	± 31%	± 10%	± 38%
Coalhurst	± 10%	± 17%	± 10%	± 43%
Lethbridge	± 10%	± 28%	± 10%	± 61%
Vauxhall	± 10%	± 19%	± 10%	± 24%

the order of 1 per cent. As can be seen from the data in Table 5, obtaining a level of accuracy equal to ± 10 per cent of the mean basal area of these minor species involves a greatly increased sampling intensity. It was not possible to calculate the sampling intensity required when using the loop method, but the data suggested that the figures involved would be very large.

By using the number of samples shown in Table 4 for the line interception and vertical point methods in combination with the variance of secondary species for the same sampling intensities, the bounds can be determined within which the estimate of the mean of secondary species will fall when obtained by these sampling intensities.

This procedure was followed to obtain the results in Table 6. Thus, when using the line interception method at the intensities shown in Table 4, an estimate of basal area of the dominant grass species is obtained which is within ± 10 per cent of the true mean ($P = 0.05$). Using the same sampling intensities on minor species, it is seen that the estimate of basal area obtained may vary from the true mean density by from ± 17 per cent to ± 31 per cent. With the point method the variation is even

TABLE 7.—COMPARISON OF COEFFICIENTS OF VARIATION AT FOUR SITES USING THREE METHODS BASED ON BASAL AREA OF THE DOMINANT SPECIES AT EACH SITE

Site	Line Interception	Point Quadrat	Loop Method
	Coef. of var. %	Coef. of var. %	Coef. of var. %
Stavely	26.87	21.97	40.97
Coalhurst	27.90	20.59	53.34
Lethbridge	15.21	12.61	15.80
Vauxhall	30.69	25.18	29.18

greater, with the mean basal area estimate of minor species varying from their true mean by from ± 24 per cent to ± 61 per cent depending upon the vegetative type.

This emphasizes an aspect of sampling which appears common to all ecological sampling. That is, a suggested sampling intensity may give reasonably accurate information for the whole or a major portion of the whole, but may not give very accurate information about an individual species or group of species which make up a relatively small part of the whole.

The coefficients of variation of basal areas of dominant species determined by each method at each site are listed for comparison in Table 7. At the Stavely and Coalhurst sites the high coefficients of variation shown for the loop method indicate that the method is of little use in these vegetative types. Basal area data could be satisfactorily obtained using either the line interception or point method. At Lethbridge and Vauxhall the coefficients of variation show that all methods measured the basal area of the dominant species in a satisfactory manner. In general the loop method gave the most variable data, the point method least, with the line interception method intermediate between the point and loop methods.

Thus, in a type of cover whose dominant species consists largely of *Bouteloua gracilis* (H. B. K.) Lag. or a grass of similar growth habit, the loop method appears very useful. Basal area data obtained are nearly as consistent as that obtained by other methods. However, the method gives extremely variable data when used to measure basal area of a single stemmed species such as *Agropyron smithii* Rydb., or an open bunch-type such as *Danthona parryi* Scribn. In this study, the line interception method was found to be most time-consuming; it detected more species than either the loop or point methods, and gave data which were only slightly more variable than those obtained by the point method. The point method gave the least variable data, detected species present in a satisfactory manner and, in general, seemed most useful in characterizing the vegetation in the range types studied.

ACKNOWLEDGEMENTS

The writer is indebted to F. A. Branson and B. Ostle, of Montana State College, and to R. W. Peake and A. F. Rayment, of the Experimental Farm, Lethbridge, Alberta, for encouragement and aid throughout the course of this study.

REFERENCES

1. Canfield, R. H. Application of the line interception method in sampling range vegetation. J. Forestry 39 : 388-394. 1941.
2. Canfield, R. H. Measurement of grazing use by the line interception method. J. Forestry 42 : 192-194. 1944.
3. Clarke, S. E., and J. A. Campbell. An ecological and grazing capacity study of the native grass pastures of southern Alberta, Saskatchewan, and Manitoba. Can. Dept. Agr. Tech. Bull. 44. 1942.
4. Coupland, R. T. Ecology of mixed prairie in Canada. Ecology Monogr. 20 : 271-315. 1950.
5. Cowling, R. W. Annual report. Pacific Northwest Forest and Range Expt. Sta., Portland, Ore. 1953.
6. Crocker, R. L., and N. S. Tiver. Survey methods in grassland ecology. J. Brit. Grassland Soc. 3 : 1-26. 1948.
7. Ellison, L. A comparison of methods of quadrating short grass vegetation. J. Agr. Research 64 : 595-614. 1942.
8. Goodall, D. W. Some considerations in the use of point quadrats for the analysis of vegetation. Aust. J. Sci. Research Series B. 5 : 1-41. 1952.
9. Hanson, H. C. A comparison of methods of botanical analysis of the native prairie in western North Dakota. J. Agr. Research 49 : 815-842. 1934.
10. Hanson, H. C. Ecology of the grassland. II. Botan. Rev. 16 : 283-360. 1950.
11. Hormay, A. L. Getting better records of vegetative changes with the line interception method. J. Range Management 2 : 67-69. 1949.
12. Levy, E. B., and E. A. Madden. The point method of pasture analysis. New Zealand J. Agr. 46 : 267-279. 1933.
13. Moss, E. H., and J. A. Campbell. The fescue grassland of Alberta. Can. J. Research, *C*, 25 : 209-227. 1947.
14. Parker, K. W. A method of measuring trend in range condition on national forest ranges. Forestry Service, U.S. Dept. Agr. Multilithed. 1951.
15. Savage, D. A. The line transect method. An improved method of studying native range vegetation. U.S. Dept. Agr. Mimeographed. 1940.
16. Sharp, L. R. Evaluation of the loop procedure of the 3-step method in salt desert shrub type of southern Idaho. J. Range Management 7 : 83-88. 1954.
17. Short, L. R. New equipment for the 3-step method. J. Range Management 6 : 184-186. 1953.
18. Tinney, F. W., O. S. Aamodt, and H. L. Algren. Preliminary report of a study of methods used in botanical analyses of pasture swards. J. Am. Soc. Agron. 29 : 835-840. 1937.
19. Torrie, J. H., H. L. Shands, and B. D. Lieth. Efficiency studies of types of design with small grain trials. J. Am. Soc. Agron. 35 : 645-661. 1943.
20. Whitman, W. C., and E. I. Siggeirsson. Comparison of line interception and point contact methods in the analysis of mixed grass range vegetation. Ecology 35(4) : 431-436. 1954.

STUDIES ON SUNFLOWER RUST

I. SOME SOURCES OF RUST RESISTANCE[1]

ERIC D. PUTT[2] AND W. E. SACKSTON[3]

[Received for publication July 16, 1956]

ABSTRACT

Sunflowers (*Helianthus annuus* L.) and sunflower rust (*Puccinia helianthi* Schw.) are both apparently native to North America. Although sunflowers were used by North American Indians, they were first grown on a large scale as a source of edible oil in Russia. Their cultivation has since spread throughout the world, with the largest acreages in eastern Europe and South America. The rust is present wherever the crop is grown, and has been a limiting factor in production in some areas.

Beacon, the first rust-resistant sunflower variety to be grown commercially, was produced in Manitoba and released to growers in 1955. It was derived from a single resistant plant which originated from a natural cross with wild sunflowers near Renner, Texas. Resistant plants have since been found in four other accessions from the Renner area, and in one from College Station, Texas. Varying degrees of rust resistance occur in selections from these sources, which have been carried through several selfed generations. Crossing studies have shown that resistance is dominant. Tests in South America indicate that lines derived from the Canadian material are resistant to rust in Peru and Argentina.

INTRODUCTION

Before 1955, no rust-resistant variety of sunflowers was available to growers in any country. In December, 1954, the rust-resistant variety "Beacon", produced in Manitoba, was licensed for distribution by the Canada Department of Agriculture. In 1955, it was grown on approximately 15,000 acres in the southern portion of the Red River Valley in Manitoba.

It is the purpose of this paper to present the background information and the methods used in the sunflower breeding program in Manitoba, and to discuss in greater detail than was possible in the preliminary note (26), the materials being used in the current effort to discover rust resistance and incorporate it into sunflower varieties suitable for Western Canada. Studies on the inheritance of rust resistance, and other phases of our research program, will be reported in subsequent papers in this series.

HISTORICAL

Sunflowers (*Helianthus annuus* L.) were found in North America by the earliest explorers and settlers. They were introduced into Spain, presumably from Mexico, and into France and England from the eastern seaboard and Canada. The plants, grown as ornamentals, were described by herbalists in Europe in the late sixteenth century (11, 12). Although sunflower seeds were used for food, as well as for oil for cosmetic and medicinal purposes, and as a source of dye, by North American Indians and early settlers, it was not until the nineteenth century, in Russia, that they were extensively cultivated for the food value of the seed (12, 13). The success of the crop in Russian and eastern Europe led to its introduction

[1] Joint contribution from the Forage Crops Division, Experimental Farms Service, and the Botany and Plant Pathology Division, Science Service (Contribution No. 1551), Canada Department of Agriculture, Ottawa, Canada.

[2] Agrostologist, Experimental Farm, Morden, Manitoba.

[3] Plant Pathologist, Plant Pathology Laboratory, Winnipeg, Manitoba.

and trial in North Africa, India, China, South Africa, Australia, New Zealand, and North and South America. There was considerable interest before 1930 in sunflowers as a forage and silage crop to substitute for corn in parts of the United States and Canada where corn did not grow well (34, 16, 15). Sunflowers as a seed crop were introduced into Canada by the Mennonite immigrants from Russia late in the nineteenth century. They were grown on a small scale in farm gardens, and the seed was roasted and used as a confection.

Sunflower seed production on a commercial scale in Canada started in 1943, as part of a government-sponsored effort to produce edible vegetable oils to relieve the war-time shortage. Between 4,000 and 5,000 acres in Manitoba and Saskatchewan were sown to short varieties suitable for combine harvesting. The acreage increased rapidly in subsequent years, and production shifted entirely to southern Manitoba. A producer-cooperative was formed to build a processing plant, which started operations in 1946. The availability of processing facilities gave further impetus to sunflower growing, so that by 1949 the crop was sown on 60,000 acres (23). Even this acreage was small, compared to world totals (exclusive of the U.S.S.R.) of 5,000,000 to 7,000,000 acres, largely in eastern Europe and South America (3), and over 8,000,000 acres in the U.S.S.R. in some years (3, 1).

Sunflower growing in Manitoba has been confined largely to the southern portion of the Red River Valley. In 1949, when the acreage reached its peak, sunflowers were sown on about 10 per cent of the total area in 30 townships. On some farms, sunflowers were sown repeatedly in the same fields for several years. Such intensive cultivation of a single crop is ideal for the development of plant diseases. The most destructive of the diseases that developed was rust.

Sunflower rust, *Puccinia helianthi* Schw., was first described in 1822 from specimens collected in southeastern United States (5, 6). It has since been reported throughout the United States, Canada, and Mexico in North America; Bermuda, Cuba, Dominican Republic, Guatemala, in Central America and West Indies; 16 countries in Europe; China, Israel, Japan, Korea, and U.S.S.R. in Asia; 9 countries throughout Africa; 4 states in Australia (2, 14, 19, 20); Argentina (10), Peru (7), Chile[1] and Uruguay[2]. Heavy infections by rust, and consequent severe losses, have been reported from Russia (9), Argentina (18), Kenya (19), Rumania (31), South Africa (8), Canada (27) and Peru[3].

Sunflower rust is eu-autoecious. Teliospores formed in the fall in Manitoba survive the winter on host debris, germinate in the spring, and produce sporidia which infect young seedlings. The earliest pycnial and aecial infections are usually found on volunteer seedlings among debris from the previous crop, and along roadsides. Aecial infections were observed on volunteer seedlings by mid-June in 1951, the year of the worst sunflower rust outbreak ever recorded in Manitoba. Infection spreads to cultivated sunflowers, which are usually sown about mid-May.

[1] Sackston, W. E. Report on sunflower disease survey from Santiago to Puerto Montt, Chile, in March, 1954. *Unpublished.*

[2] Fischer, J. G. Rust on sunflowers in Uruguay. *Personal communication.* 1955.

[3] Swanson, A. F. Rust damage to sunflowers in Peru. *Personal communication.* 1954.

As sunflower rust outbreaks are usually local in origin, crop rotation, field sanitation, and the maximum possible isolation from other fields of the current year and from rusted debris of the previous year, all tend to delay disease development and to minimize damage to the crop. Such measures are only palliative, however, and severe infection and loss may occur in spite of them.

Control of sunflower rust by fungicides has succeeded in experimental plots (28, 30). For best results, the plants should be sprayed or dusted at the flowering stage, when they are about 4 feet tall. Ground application is difficult without damaging such tall plants with the equipment ordinarily available in sunflower growing areas. Aeroplane application is expensive and impractical, because most fields are relatively small.

The most effective method of controlling rust of field crops is the production of resistant varieties. Various workers have been seeking rust-resistant sunflowers for many years. The South American variety Kaeurpher was reported in 1920 to be resistant in Michigan, when Mammoth Russian was practically killed by rust (33). No further work with the variety has been published, and it is not grown now in South America; so its resistance may have been more apparent than real. Vinall (35) quoted Russian reports that *Helianthus argophyllus* was rust resistant, and that crosses with cultivated sunflowers segregated to give 1 resistant to 3 susceptible plants in the F_2 generation. An abstract of a report from the Manfredi Station in Argentina (4) indicates that, although *H. argophyllus* was susceptible to rust, crosses between it and cultivated sunflowers, and between *H. cucumerifolius* and cultivated sunflowers, gave some resistant plants in the hybrid populations. Lebedeva in Russia (17) found that dwarf and early-maturing varieties were more susceptible to rust than tall and late varieties. Eremayeva and Karakulin (9) obtained some inconclusive evidence of varietal and individual plant resistance among the varieties they tested. Scherbak (32), also working in the U.S.S.R., reported failure to get rust resistance by selfing resistant individuals and by inter-specific crosses. He inbred certain varieties that had some field resistance, presumably by growing them in isolation, and found that in 3 years the original resistance was markedly increased.

Sunflower varieties produced recently in the U.S.S.R. have not been tested in Manitoba nor have the interspecific hybrids recently reported from Argentina. All the available varieties from Europe and South America, however, have proved to be susceptible to rust. Until 1949, no source of rust resistance was available for use in the sunflower breeding program in Manitoba. In that year, one resistant plant was found in an experimental plot; other resistant plants were discovered later (26). Genetical studies with some of the Canadian material (25) indicate that the factors for rust resistance in it are dominant and therefore different from the recessive characters reported by Scherbak in the U.S.S.R. (32).

METHODS

Sunflowers are cross-pollinated by insects. In order to make controlled crosses, it is possible to emasculate some of the florets on a head and remove the rest, before applying pollen from the desirable male parent

(22). This procedure has been followed in some studies but it is extremely time-consuming. An alternative method is to cover the heads with a bag of some suitable material to prevent chance pollination, apply pollen of the desired male parent, and pick out the hybrid from the selfed seed in the next generation on the basis of vigour or other characters (22, 24). Bagging is the usual method employed in selfing individual plants (22). Some sunflower selections may be selfed readily, and produce as many as 200 to 300 seeds per head; others are relatively self sterile. Brushing the bagged heads usually improves seed setting.

The methods used in rust inoculations and in making the rust readings reported here varied with the nature and location of the tests. Rust ratings on early tests in naturally infected field plots at Altona and Morden were based on percentage infection. Plants with low percentages of leaf area rusted were rated resistant, while those with high percentages were considered susceptible. In later tests, one plant of the extremely susceptible inbred line S37-388 was sown at one end of each plot of 15 test plants, and was inoculated with rust. As the infections spread along the entire rows of the susceptible check varieties Sunrise and S-37-388, spaced at intervals throughout the plots, it was assumed that plants free of rust or only lightly infected in test rows had been exposed to rust, and were resistant. The inoculum was collected from naturally infected sunflower leaves from plots or farm fields. Ratings in Peru were made on the basis of reaction type rather than percentage infection, in field plots inoculated by dusting with spores from naturally infected leaves collected in a farm field. Greenhouse and field ratings at Winnipeg were based on reaction type of inoculated plants.

Field plots at Winnipeg were inoculated in one of two ways. Some were sprayed with suspensions of urediospores in water, at concentrations of 250,000 spores per ml. One litre of spore suspension was sprayed on 60 feet of row when plants were about 3 feet tall. When the plants were 4½–5 feet tall, in the bud or flower stage, 1 litre was used for 30 feet of row, approximately equivalent to 100 gallons of spray per acre. Other plots were inoculated with urediospores diluted with talc 1 : 10 (sometimes 1 : 50), applied with a duster.

Inoculations at Morden were made by applying urediospores, diluted approximately 1 : 50 with bentonite clay, with a duster. Field inoculations at Winnipeg and Morden were usually made in the evening to provide favourable conditions for spore germination and infection.

SOURCES OF RESISTANCE

The sources of seed and the pedigrees of the six sunflower accessions with apparent rust resistance discussed in this paper are indicated in Table 1.

Morden Accession 953-88

Accession 953-88 was grown at Altona, Manitoba in 1949 from seed obtained from open-pollinated F_1 plants of the natural cross Sunrise × Texas Wild Annual at Renner, Texas. One plant in the F_2 population, like wild annual sunflowers in appearance, bore only a few small pustules of rust when adjacent susceptible material had up to 50 per cent infection.

TABLE 1.—PEDIGREES OF THE SUNFLOWER ACCESSIONS POSSESSING RESISTANCE TO RUST

Morden accession no.	Source of seed	Pedigree of the seed
953-88	R. Kalton Renner, Texas	From open pollinated F_1 plants of the natural cross Sunrise × Texas Wild Annual
953-102	Renner, Texas	From open-pollinated F_1 plants of the natural cross California Oilseed × Texas Wild Annual
307	E. Collister Renner, Texas	From open-pollinated Hopi plants
306	E. Collister Renner, Texas	From open-pollinated F_1 plants of the cross Hill Sunrise × Wild
355	M. L. Kinman College Station, Texas	From one rust-free, open-pollinated plant of "Red 6"
403	E. Collister Renner, Texas	Advance Hybrid (S37-388 × Sunrise)

This plant was self-pollinated. Seedlings in its progeny proved resistant when inoculated in the greenhouse with a field collection of rust. Other plants of the progeny were grown in the field in 1950 and were used as male parents in crosses with rust-susceptible inbred lines of desirable agronomic (commercial) type. The F_1 generation of these crosses contained plants which were only lightly infected in the field in 1951, in the severest rust outbreak recorded in Canada.

Some of the F_1 plants were backcrossed to the commercial parent, others were used in three-way crosses with a second commercial type inbred line, and the rest were self-pollinated. The seed obtained was sown in Chile during the winter of 1951-52, and the resulting plants were self-pollinated.

About 1500 single-plant progenies were grown at Morden in 1952 in the F_3 generation from the original crosses, or as the first selfed generation from the backcrosses and three-way crosses. One hundred and thirty-three progenies, containing 8 to 45 plants each were selected on the basis of agronomic type. All the plants were scored for rust infection on the scale of Peterson *et al.* (21). Mean infection of the progenies ranged from 0.9 to 42.0 per cent, with an average for all plants in the 133 selected lines of 9.6 per cent. The mean infection of 24 rows of susceptible check varieties ranged from 8.2 to 42.8 per cent, but the average infection for all plants was 26.9 per cent.

Three hundred and twenty plants with 5 per cent or less rust infection were selected in the 133 progenies and were self-pollinated. Rust reactions of the resulting progenies were determined in the greenhouse during the winter of 1952-53. Three isolates of rust from different localities were used to inoculate different groups of one to five seedlings of each progeny.

TABLE 2.—SUMMARY OF SEEDLING REACTION TO THREE RUST ISOLATES OF 320 PROGENIES DERIVED FROM CROSSES OF SUSCEPTIBLE SUNFLOWERS × MORDEN 953-88

Progeny reaction to individual rust isolates	Number of progenies	Percentage of total
Resistant to three isolates	26	8.1
Resistant or segregating	91	28.4
Resistant, segregating, or susceptible	109	34.1
Susceptible to three isolates	94	29.4

The results are summarized in Table 2. The uniform resistance of 8.1 per cent of the progenies to all three rust isolates were very encouraging, as was the occurrence of at least some resistant plants in 70.6 per cent of the progenies tested.

Other plants of the same progenies were grown in Chile at the same time as the green house tests were being made at Winnipeg. Sixty-one plants in 26 progenies in the field in Chile were selected on the basis of the greenhouse results, and allowed to interpollinate. The bulked seed, increased and tested in the field in Canada, was licensed in December 1954 as the rust-resistant variety, Beacon. This variety yields approximately as well as the commonly grown hybrid variety, Advance, when rust infection is light, but greatly outyields Advance when rust infection is heavy.

The progenies composing Beacon, and related selections, have been carried into advanced generations by continuous self-pollination on a single-plant basis. More than 400 lines bearing the 953-88 source of resistance were grown in 1954. In 69 lines, each with at least 5 plants, and most with 10 to 15 plants, rust infection in the field did not exceed 1 per cent of any plant. In 192 lines, rust infection did not exceed 5 per cent on any plant. Infection in susceptible check material ranged from 10 to 90 per cent.

Morden Accession 953-102

Accession 953-102 was grown at Morden in 1950 from seed collected from open-pollinated F_1 plants of the natural cross California Oilseed × Texas Wild Annual, at Renner, Texas. Open-pollinated seed was obtained from one plant with low rust infection in the F_2 generation. The progeny of this individual included one large plant, designated 953-102-1-1, which bore only two minute pustules in the field in 1951, when nearby susceptible sunflowers were practically destroyed by rust.

Some of the open-pollinated seed produced by this plant was used to determine seedling reaction to rust in the greenhouse, given in Table 3; some of it was sown in the greenhouse, and the resulting plants were grown to maturity and self-pollinated; some was sown in the field in Chile, and the plants were self-pollinated. Seed from 58 of the selfed plants was sown at Morden in 1952.

TABLE 3.—REACTION TO SUNFLOWER RUST OF PLANTS DERIVED FROM MORDEN ACCESSION 953-102

Selection	Generation[1]	Date of test	No. of progenies	Seedling reaction in greenhouse		Adult plant reactions Percentage rust in field			
				Res.	Sus.	0	Tr. to 1%	2 to 10%	over 10% (20%-70%)
953-102-1-1	O.P.	1951-52	1	6[2]	5[2]	—	—	—	—
953-102-1-1-22	S_1	1952	1	—	—	4	11	—	—
953-102-1-1-22	S_2	1952-53	12	229[2]	38[2]	—	—	—	—
953-102-1-1-22	S_2, S_3	1953	21	—	—	221	11	1	1
953-102-1-1-22	S_2, S_3	1953-54	4	6[2]	18[3]	—	—	—	—
953-102-1-1-22	S_2, S_3	1954	3	—	—	10	7	0	0
953-102-1-1-22	S_4	1954	5	—	—	11	21	0	0
953-102-1-1-41	S_1	1952	1	—	—	7	8	0	0
953-102-1-1-41	S_2	1952-53	9	167[2]	46[2]	—	—	—	—
953-102-1-1-41	S_2, S_3	1953	6	—	—	38	5	1	0
953-102-1-1-41	S_3, S_4	1953-54	4	23[3]	0[3]	—	—	—	—
953-102-1-1-41	S_4	1954	2	—	—	12	13	1	0
953-102-1-1-54	S_1	1952	1	—	—	8	3	0	0
953-102-1-1-54	S_2	1952-53	1	27[2]	0	—	—	—	—
953-102-1-1-54	S_2, S_3	1953	3	—	—	35	0	0	0

[1] O.P. = open pollinated
S_1, S_2, S_3, S_4 = first, second, third, fourth selfed generation
[2] Inoculated with three single-pustule isolates
[3] Inoculated with a bulk collection of rust from the field.

Two of the 58 progenies, each containing 15 plants, were outstandingly resistant to rust. The first of these two lines, 953-102-1-1-22, had 4 plants free of rust, 10 plants with only one or a few pustules, and 1 plant with a few pustules on most leaves. The second line, 953-102-1-1-41, had seven plants free of rust, 5 plants with only one or a few pustules, and 3 plants with a few pustules on each leaf. Several other progenies also contained some plants on which no rust was found. Nearby susceptible check varieties had from 20 to 80 per cent rust infection.

Reaction to rust of seedlings from 21 selfed progenies of the two outstanding lines is given in Table 3. All the seedlings in 10 of the progenies were resistant in all tests. In the remaining 11 progenies, segregation for resistance and susceptibility occurred in the tests with one, two, or three of the rust isolates used, but in no test were all the seedlings susceptible.

Selections derived from the 953-102 line continue to show a high degree of resistance to rust in the field. A total of 388 plants with this parentage were grown at Morden in 1953 and 1954. Three hundred and twenty-seven plants were free of rust; 57 had less than 1 per cent; 3 had between 1 and 5 per cent; and 1 plant had 20 per cent infection. The data in Table 3 indicate a striking difference between the reaction in the seedling stage of the 953-102-1-1-22 and 953-102-1-1-41 selections to a field collection of rust in experiments in 1953-54. No such difference in reaction of the

TABLE 4.—REACTION TO SUNFLOWER RUST OF PLANTS DERIVED FROM SEVERAL MORDEN ACCESSIONS

Selection	Generation	Date of test	No. of progenies	Seedling reaction in greenhouse		Adult plant reactions Percentage rust in field			
				Res.	Sus.	0	Tr. to 1%	2 to 10%	over 10% (20%-70%)
307	O.P.	1952-53	1	9	17	—	—	—	—
307	S_1	1953	9	—	—	12	41	22	0
Sunrise	—	1953	—	—	—	0	0	8	0
307	S_2	1953-54	7	45[1]	18[1]	—	—	—	—
307	S_2	1954	11	—	—	6	71	42	6
S37-388	—	1954	—	—	—	0	0	0	12
306	O.P.	1952-53	1	12	12	—	—	—	—
306	S_1	1953	5	—	—	6	16	17	0
S37-388	—	1953	—	—	—	0	0	8	3
355	S^1	1953-54	1	5	0	—	—	—	—
355	S_1	1954	1	—	—	67	59	15	11
Sunrise	—	1954	—	—	0	0	0	0	12
355	S_2	1954-55	4	39[2]	13	—	—	—	—
403-3	O.P.	1952-53	1	8	16	—	—	—	—
403-3	S_1	1953	6	—	—	28	12	12	2
S37-388	—	1953	—	—	—	0	0	8	3
403-4	O.P.	1952-53	1	15	12	—	—	—	—
403-4	S_1	1953	10	—	—	45	25	21	8
Sunrise	—	1953	—	—	—	0	0	8	0

[1] Reactions based on pustule type of adult plants grown in field plots artificially inoculated at La Molina, Peru.

[2] Inoculated with a bulk collection of rust from the field. Resistant reactions were dominant, but susceptible type pustules were also present on 34 of the 39 plants.

two lines has been observed in the field. As the first-named line is of a more desirable agronomic type than the second, it is being used extensively in further breeding work. Plants derived from this line have proved resistant to rust not only in Manitoba, but also in the field in Peru, and in greenhouse and field tests in Argentina.

Morden Accession 307

Accession 307 was grown at Altona, Manitoba, in 1952 from seed produced by open-pollinated Hopi plants at Renner, Texas. Hopi is a variety of sunflower cultivated by the Hopi Indians of Arizona (11). The resulting population contained one plant, 307-1, with very light rust infection, from which open-pollinated seed was harvested. The reaction to rust of seedlings from part of this seed is indicated in Table 4. The rest of the seed was sown in Chile, and the plants produced were self-pollinated. The rust reaction of the selfed progenies in the field at Morden is indicated in Table 4. Although rust infection on the check variety Sunrise ranged only from 2 to 6 per cent, the low infection rating of most plants of the 307-1 progenies was assumed to be evidence of resistance.

Rust reactions of adult plants of the second and third selfed generations in the field at La Molina, Peru, and Morden, respectively, are given in Table 4.

Morden Accession 306

Accession 306 was grown at Altona in 1952 from seed collected from open-pollinated F_1 plants of the cross Hill Sunrise × Wild at Renner, Texas. One open-pollinated plant in the plot had only light rust infection. Part of the seed was sown in the greenhouse and the rust reaction of the seedlings was determined (Table 4); the rest of the seed was sown in Chile, and the plants were self-pollinated. Rust reactions of plants of five of the selfed progenies in the field at Morden are given in Table 4.

Morden Accession 355

Accession 355 was grown at Morden in 1953 from seed of one rust-free open-pollinated plant of "Red 6" produced at College Station, Texas. One large, vigorously branching, purple-flowered plant in the resulting population was rust-free. It was self-pollinated. The reaction to rust of seedlings of the first and second selfed generations in the greenhouse, and adult plants of the first selfed generation in the field, are given in Table 4. The heaviest infection observed on the "355" material was 30 per cent, while on the susceptible checks it ranged from 40 to 80 per cent.

Morden Accession 403

Accession 403, grown at Morden in 1952, consisted of 5 lb. of Advance Hybrid seed (S37-388 × Sunrise) produced at Renner, Texas. The plot at Morden contained off-type plants characterized by a vigorous branching habit and, in many cases, purple flowers. These plants were assumed to be natural crosses of the female parent, S37-388, with wild *Helianthus annuus* L. of the Renner area. Open-pollinated seed was harvested from four of the off-type plants which were lightly infected with rust, in contrast to the heavily rusted Advance plants around them. Two of the four progenies produced seedlings which were all susceptible to rust in greenhouse tests; the reaction to rust of seedlings of the other two progenies is given in Table 4. Plants of the lines 403-3 and 403-4 were grown and self-pollinated in Chile, and the seed produced was sown at Morden. The rust reaction of the 16 selfed progenies is given in Table 4. Each progeny contained at least one plant free of rust. Rust infections on adjacent rows of the susceptible check variety, Sunrise, were low, ranging from 2 to 6 per cent.

CONCLUSIONS AND DISCUSSION

All the accessions in which rust-resistant plants occurred came from Texas, and all but Morden 355 came from Renner. It is assumed that one parent of the natural crosses in each of the accessions 953-88, 953-102, 306, and 403, was the wild *H. annuus* of the Renner area. This assumption is based on the vigorous branching and the purple flower colour, characteristic of the wild species, which appeared in the segregating populations; and the occurrence of only this wild annual sunflower species in the Renner region.

The Sunrise parent of accessions 953-88 and 306, and the S37-388 parent of accession 403, are susceptible to rust. It has not been possible to obtain definite information on the California Oilseed parent of accession 953-102. Other commercial sunflowers in California are susceptible to rust, and no reports of rust resistance in commercial varieties there have been received, so it may be assumed that this variety also is rust susceptible. It appears, therefore, that the rust resistance found in these four accessions came from the wild *H. annuus* growing in the vicinity of Renner, Texas.

The Hopi plant from which accession 307 was derived was open-pollinated in the Renner area. It is possible, therefore, that the resistance encountered in this accession may also have resulted from natural crossing with wild *H. annuus*.

Seed of accession 355 came from M. L. Kinman, College Station, Texas, but the open-pollinated plant on which it was produced was grown from seed supplied by C. B. Heiser of Bloomington, Indiana. As both workers had selections of the wild *H. annuus* of Texas growing in their nurseries, the resistance of accession 355 might have resulted from natural crossing with the wild species.

H. annuus grows wild over much of the central Great Plains area of North America. It, or a subspecies, occurs commonly in the main sunflower growing area of Canada, and crosses readily with cultivated varieties. No evidence of rust resistance in the Canadian wild form has yet been found. The resistance discovered to date, with the possible exception of that in accessions 307 and 355, seems to have come from the Texas ecotype of wild *H. annuus*, and more specifically, from the population occurring in the vicinity of Renner, which may prove to be a reservoir of genes for rust resistance. Extensive sampling of wild sunflowers throughout the central plains is required, to determine the geographical distribution of such genes.

Work is in progress to determine if the rust resistance of accessions 953-88, 953-102, and 307-1 is controlled by the same genes, and also to determine the inheritance of resistance in crosses of each of these accessions with susceptible sunflowers. Additional rust resistant accessions are being included in these investigations as time and facilities permit.

ACKNOWLEDGEMENTS

The authors acknowledge their indebtedness to the many individuals who have contributed to these studies at various times. They include: W. A. Russell, while at the Experimental Farm, Morden, Man., 1949-52; A. M. Brown, Plant Pathology Laboratory, Winnipeg, Man., 1949-50; A. F. Swanson while with Institute of Inter-American Affairs, Lima, Peru; Emilio Rojas, Agricultural Experiment Station, La Molina, Peru; Mario Astorga, Ministerio de Agricultura, Santiago, Chile; Walter Kugler, Estacion Experimental, Pergamino, Argentina; R. Kalton, while at the Texas Research Foundation, Renner, Texas, and E. Collister, of the same institution; and M. L. Kinman, U.S. Department of Agriculture, College Station, Texas.

REFERENCES

1. *Anonymous.* Vegetable oils and oilseeds. Commonwealth Economic Committee. H. M. Stationery Office, London. 1950.
2. *Anonymous. Puccinia helianthi* Schw. on Helianthus. Commonwealth Mycological Institute Map No. 195. Revised to January 2, 1950.
3. *Anonymous.* Food and agricultural statistics. F. A. O. Yearbook. 8 (1) : 87. 1954.
4. *Anonymous.* Progreso realizada en las investigaciones agricolas durante el ano 1953. IDIA. 1954 (3-4-5) 1954. *In* Plant Breed Abstr. 25 : 152-156. 1955.
5. Arthur, J. C. Manual of the rusts of the United States and Canada. Purdue Research Foundation, Lafayette, Indiana. 1934.
6. Bailey, D. L. Sunflower rust. Minn. Agr. Expt. Sta. Tech. Bull. 16. 1923.
7. Bazan de Segura, C. Principales enfermedades de las plantas en el Peru. Estac. Expt. Agr. La Molina, Bol. 51. 1953.
8. Doidge, E. M. South African rust fungi. Part V. Bothalia 4 (4) : 895-918. 1948. *In* Rev. Appl. Mycol. 29 : 333. 1950.
9. Eremayeva, A. M., and B. P. Karakulin. (Observations on the sunflower rust at the Lower Volga Regional Agricultural Experiment Station). Morbi Plantarum, Leningrad, 18(1-2) : 11-30. 1929. *In* Rev. Appl. Mycol. 8 : 791-793. 1929.
10. Godoy, Ernesto F. Epifitia de roya del girasol en la Argentina. IDIA. Mayo de 1953 : 2-8. 1953.
11. Heiser, Charles B., Jr. A "new" cultivated sunflower from Mexico. Madrono 8(7) : 226-229. 1946.
12. Heiser, Charles B., Jr. The sunflower among the North American Indians. Proc. Am. Phil. Soc. 95(4) : 432-448. 1951.
13. Heiser, Charles B., Jr. The origin and development of the cultivated sunflower. Amer. Biol. Teacher 17(5) : 161-167. 1955.
14. Hendrickx, F. L. (Annotated list of Congolese Fungi.—I.). Publ. Inst. nat. Etude agron. Congo belge 1(2) : 125-144. 1944. *In* Rev. Appl. Mycol. 30(4) : 195-196. 1951.
15. Hulbert, H. W., and J. H. Christ. Growing sunflowers for silage in Idaho. Idaho Agr. Expt. Sta. Bull. 141. 1926.
16. Jensen, I. J. Sunflowers under irrigation in Montana. Montana Agr. Expt. Sta. Bull. 162. 1923.
17. Lebedeva, L. A. (Mycophenological observations made during the summer of 1927 on cultivated plants at Saratoff Regional Agricultural Experiment Station and in its immediate vicinity). (Materials for Mycol. and Phytopath., Leningrad). 6(1) : 227-238. 1927. *In* Rev. Appl. Mycol. 7 : 297-298. 1928.
18. Muntanola, Maria. Sobre la presencia de la roya del girasol (*Puccinia helianthi* Schw.) en Tucuman. Rev. Agron. Noroeste Argent. 1(2) : 213-215. 1954. *In* Rev. Appl. Mycol. 34(12) : 789. 1955.
19. Nattrass, R. M. Annual report of the senior plant pathologist. Rept. Dept. Agr., Kenya, 1948 : 95-98. 1950. *In* Rev. Appl. Mycol. 30(6) : 308-309. 1951.
20. Noronha, E. de A. (Phytopathological notes, from August, 1948 to May, 1949.) Agron. angol., 2 : 37-44. 1949. *In* Rev. Appl. Mycol. 29(8) : 408. 1950.
21. Peterson, R. F., A. B. Campbell, and A. E. Hannah. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can. J. Research, *C*, 26 : 496-500. 1948.
22. Putt, Eric D. Investigations of breeding technique for the sunflower (*Helianthus annuus* L.). Sci. Agr. 21 : 689-702. 1941.
23. Putt, Eric D. Sunflower seed production. Co-op. Vegetable Oils Ltd., Altona, Man. 1952.
24. Putt, Eric D. Note on the value of cotton and paper bags for self-fertilizing sunflowers. Can. J. Agr. Sci. 34 : 326-327. 1954.
25. Putt, Eric D., and Emilio Rojas M. Field studies on the inheritance of resistance to rust in the cultivated sunflower (*Helianthus annuus* L.). Can. J. Agr. Sci. 35 : 557-563. 1955.
26. Putt, Eric D., and W. E. Sackston. Rust resistance in sunflowers (*Helianthus annuus* L.). Nature 176(4471) : 77. 1955.
27. Sackston, W. E. Diseases of oilseed crops in Manitoba. Proc. Can. Oilseeds Conference 1952 : 60-61. 1952.

28. Sackston, W. E. Preliminary investigations on the chemical control of sunflower rust. (Abstr.). Proc. Can. Phytopathol. Soc. 21 : 17-18. 1953.
29. Sackston, W. E. Forms and races of *Puccinia helianthi*. (Abstr.). Phytopathol. 46 : 25. 1956.
30. Sackston, W. E., and Eric D. Putt. Rust as a factor in sunflower production. (Abstr.). Phytopathol. 46 : 25. 1956.
31. Savalescu, T. (Rumania. Phytopathological events during the year 1941.) Interie. Bull. Plant Prot. 33(2) : 17M-19M; 33(3) : 36M-39M. 1942. *In* Rev. Appl. Mycol. 21 : 404-405. 1942.
32. Scherbak, S. (Breeding the sunflower for resistance to rust.) Bull. Appl. Botan. Select., 1937. Ser. A, 21 : 67-76. 1937. *In* Rev. Appl. Mycol. 16 : 539. 1937.
33. Spragg, F. A., and E. E. Down. Rust resisting sunflowers. Mich. Agr. Expt. Sta. Quart. Bull. 2(3) : 128-129. 1920.
34. Vass, A. F. Sunflowers: Their culture and use. Wyo. Agr. Expt. Sta. Bull. 129 : 79-107. 1921.
35. Vinall, H. N. The sunflower as a silage crop. U.S. Dept. Agr. Bull. 1045. 1922.

GENETIC CHANGES IN APPLES INDUCED BY THERMAL NEUTRONS[1]

C. J. BISHOP[2]

[Received for publication August 16, 1956]

ABSTRACT

Sectorial colour changes in Cortland apples showed a marked increase over controls following irradiation of the dormant scions with 2-8 hours' exposure to thermal neutrons. This type of atomic radiation appeared to be much more effective than were x-rays in producing gene mutations. Both sectorial and complete colour sports were found, the latter being very dark red and readily distinguishable from the colour of the normal Cortland. About 5 hours' exposure with a thermal neutron dosage of about 13×10^{12} neutrons per cm^2 appeared to be the optimum treatment for this apple variety.

INTRODUCTION

The discovery of radiation-induced changes in the skin colour of apples has been reported previously by Granhall (6) who noted an increase in the amount of red blush on apples of the variety Gravenstein, and Bishop (1) who found sectorial chimaeras of both darker and lighter red colour in the variety Cortland. Neither report indicated that complete red sports had been obtained. Both were based on experiments using x-rays as the type of artificial radiation.

More recently Bishop and Aalders (3) have presented data indicating that thermal neutrons produced in apples proportionately more morphological changes of the bifurcation type than did x-rays. They attributed these to chromosomal rather than cytoplasmic effects and suggested that, in line with other evidence [Conger and Giles (5), Caldecott *et al.* (4) and Spencer *et al.* (7)], thermal neutrons may be a more effective mutagenic agent than x-rays for producing genetic changes in apples.

Preliminary results based on sectorial colour changes in the first fruits from the thermal neutron treated scions have already been reported (2). The present paper gives the data from the first sizable crop of fruit, that produced in 1955. Though the conclusions from this research are based on sectorial colour chimaeras, several unmistakable complete dark red sports have been produced by the thermal neutron treatments.

MATERIALS AND METHODS

Dormant Cortland apple scions were treated for 2, 4, 6 or 8 hours by exposure to a thermal neutron dosage of 3.9×10^{12}, 7.8×10^{12}, 11.7×10^{12}, and 15.6×10^{12} neutrons per cm^2 respectively. For comparison, other scions were treated with 4000 or 5000 roentgens of x-rays, and still others left untreated as a control. The scions were grafted by the framework method into grown trees of the varieties Red King, McIntosh and Cox Orange in the spring of 1953. Twenty-two trees were grafted with a total of 3337 scions, an average of approximately 150 scions per tree.

[1]Contribution No. 878 from the Horticulture Division, Experimental Farms Service.

[2] Superintendent, Experimental Farm, Canada Department of Agriculture, Kentville, Nova Scotia.

The thermal neutron treatments were carried out at the Brookhaven National Laboratory of the U.S. Atomic Energy Commission. The x-ray treatments were made at the Department of Biology, Acadia University, Wolfville, Nova Scotia, using equipment supplied by the Nova Scotia Research Foundation.

The fruit produced by the irradiated scions was picked when well matured, the apples from each tree of each radiation dosage being kept separate. A record was later taken of the frequency of the sectorial colour changes. Because of the difficulty in the accurate identification of complete colour sports, due to the natural variation in colour found in different locations of a tree where there are varying degrees of shade and sunlight, only those changes shown as sectorial chimaeras were recorded. No attempt was made to determine whether the colour change was toward more or less colour, as this was impossible to decide in the case of large sectors on individual apples. Multiple sectoring on single apples was considered to be a chimaeral expression of a single radiation induced change and was recorded as a single sector.

All records were taken by a single person and the boxes were checked at random without previous knowledge of the dosage.

RESULTS

A small crop of apples was produced a year after grafting, but most of the fruit was lost in a fall hurricane. In the following year (1955) there were nearly 10,000 apples, distributed as shown in Table 1.

TABLE 1.—FRUIT PRODUCTION FROM IRRADIATED CORTLAND APPLE SCIONS, 1955

Treatment	Total scions	Total apples	Flower clusters per scion	Apples per scion
Control	261	809	16.1	3.1
3.9×10^{12} N.Th.	342	2092	12.8	6.1
7.8×10^{12} N.Th.	547	2865	9.8	5.2
11.7×10^{12} N.Th.	557	1667	6.7	3.0
15.6×10^{12} N.Th.	397	701	4.7	1.8
4000r	461	673	7.6	1.5
5000r	772	1087	4.8	1.4
	3337	9894		

It is quite clear from Table 1 that with increasing amounts of radiation there was a decrease in the flower and fruit production of the scions. There was also some indication that at the rates applied the thermal neutrons may be less damaging to apple buds than the x-rays, and that the lower dosages of thermal neutrons increased the fruit set.

Both sectorial colour chimaeras and complete dark red sports were observed in the fruits on the growth from the irradiated scions. Usually these were individual apples on branches which otherwise bore normal fruit, but occasionally all the apples from a branch showed evidence of colour sectors. When this was the case, considerable variation in the width of the sectors was noted, with a few apples showing a complete colour change. In one case a sizable branch bearing all dark red fruit was located. In this instance the fruit was sufficiently dark red (approximately equal in shade of colour to the variety Macoun) so that there was no doubt that the change was genetic rather than environmental.

The frequency of the sectorial colour changes found in the apples from the irradiated scions is given in Table 2.

TABLE 2.—FREQUENCY OF SECTORIAL CHIMAERAS IN THE FRUIT FROM IRRADIATED CORTLAND APPLE SCIONS—1955 CROP

Tree No.	Treatment	Number scions	Number apples	Number sectors	Percent sectors
12-4	Control	146	407	13	3.20
12-5	Control	115	402	6	1.49
		261	809	19	2.35
14-6	3.9×10^{12} N.Th.	64	247	5	2.02
14-7	3.9×10^{12} N.Th.	215	1667	135	8.10
14-8	3.9×10^{12} N.Th.	63	178	7	3.94
		342	2092	147	7.03
14-3	7.8×10^{12} N.Th.	299	943	118	12.52
14-4	7.8×10^{12} N.Th.	173	646	84	13.00
14-5	7.8×10^{12} N.Th.	145	1276	96	7.53
		547	2865	298	10.40
12-1	11.7×10^{12} N.Th.	164	117	25	21.30
13-1	11.7×10^{12} N.Th.	207	811	96	11.84
14-1	11.7×10^{12} N.Th.	186	739	47	6.37
		557	1667	168	10.08
13-2	15.6×10^{12} N.Th.	240	307	50	16.30
13-3	15.6×10^{12} N.Th.	157	394	34	8.63
		397	701	84	11.98
12-6	4000r	131	171	7	4.09
12-7	4000r	129	286	24	8.39
12-8	4000r	102	180	3	1.67
12-9	4000r	99	36	1	2.78
		461	673	35	5.19
12-2	5000r	205	409	16	3.91
12-3	5000r	147	261	4	1.53
12-10	5000r	145	159	4	2.51
12-11	5000r	157	145	2	1.38
12-12	5000r	118	113	5	4.42
		772	1087	31	2.85

DISCUSSION

The frequency of sectorial chimaeras in the fruit was distinctly higher among those from the irradiated scions than those from the control scions (Table 2). Furthermore, it seemed that at the dosage levels used the thermal neutrons were more effective than x-rays in producing colour changes. This is in line with the previous report from this station (3) based on morphological changes, and supports the preliminary results obtained in 1954 (2).

The fact that complete dark red sports of the variety Cortland were found for the first time in the fruit from thermal neutron treated scions is further evidence that this type of radiation is genetically effective. The exact nature of this gene change is not known, as dark red colour in apples has been shown to be due to a dominant gene (8) and radiation-induced "mutations" are generally considered to be mainly destructive changes. Possibly the secondary radiation resulting from thermal neutron capture (9) is more specific in its action than x-rays and gene changes to the dominant form actually do occur. A second possibility is that the radiation has produced a dominant change through the destruction or deletion of a suppressor gene.

Considering the scion survival and growth previously reported (3) and the fruit production and percentage sectoring indicated in Table 2, 5 hours' exposure with a thermal neutron dosage of about 13×10^{12} neutrons per cm^2 would seem to be about optimum for the Cortland apple.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the co-operation of Seymour Shapiro, of the Brookhaven National Laboratory, Upton, Long Island, N.Y., in carrying out the thermal neutron treatments. G. M. Weaver, D. L. Craig, and L. E. Aalders assisted in the collection of much of the data.

REFERENCES

1. Bishop, C. J. Mutations in apples induced by x-radiation. J. Heredity 45 : 99-104. 1954.
2. Bishop, C. J. The production of bud sports in apples through the use of artificial radiation. Proc. XIVth Intern. Hort. Congr. 1956. (*In press.*).
3. Bishop, C. J., and L. E. Aalders. A comparison of the morphological effects of thermal neutrol and x-irradiation of apple scions. Am. J. Botany 42 : 618-623. 1955.
4. Caldecott, R. S., B. H. Beard, and C. O. Gardner. Cytogenetic effects of x-ray and thermal neutron irradiation on seeds of barley. Genetics 39 : 240-259. 1954.
5. Conger, A. D., and N. H. Giles. The cytogenetic effect of slow neutrons. Genetics 35 : 397-419. 1950.
6. Granhall, I. X-ray mutations in apples and pears. Hereditas 30 : 149-155. 1953.
7. Spencer, J. L., W. R. Singleton, and A. F. Blakeslee. Induced pollen lethals from seeds of *Datura stramonium* treated with thermal neutrons. Proc. Natl. Acad. Sci. (U.S.) 39 : 288-293. 1953.
8. Wilcox, A. N., and E. Angelo. Apple breeding studies. I. Fruit colour. Proc. Am. Soc. Hort. Sci. 33 : 108-113. 1935.
9. Zirkle, R. E. Components of the acute lethal action of slow neutrons. Radiology 49 : 271-273. 1947.

A STUDY OF THE GERMINATION OF BARLEY SEED TREATED TO CONTROL LOOSE SMUT[1]

J. E. MACHACEK AND H. A. H. WALLACE[2]

[Received for publication June 13, 1956]

ABSTRACT

Tests of barley seed treated by the water-soak and the Spergon method to control loose smut (*Ustilago nuda* (Jens.) Rostr.) have shown that both treatments reduce germination, the Spergon treatment being the more injurious of the two. A part of the reduction may be due to an induced dormancy, but usually the ungerminated seeds are dead.

Varieties of barley vary greatly in susceptibility to injury from treatment, but an equally great variation may occur among different lots of seed of the same variety. Susceptibility to the water-soak treatment is closely correlated with susceptibility to the Spergon treatment. Treatment with Ceresan M improves germination and there is a correlation between improvement by this treatment and reduction in germination caused by the water-soak or the Spergon treatment.

Treatment of barley seed by the water-soak and Spergon methods destroys a part of the *Alternaria* contaminant usually present in such seed, but has less effect on it than Ceresan M. Treatment of the seed by the anaerobic-incubation method as used in this study had scarcely any suppressive effect on *Alternaria*.

INTRODUCTION

Two defects of the hot-water method of treating barley seed against loose smut are its cumbersomeness and its tendency to reduce seed germination. The latter defect is of varying importance, as some lots of seed are more affected than others (5, 9). It has been suggested (2, 5, 9, 10) that the injury from hot-water treatment is indirectly due to the mechanical injury sustained by the seed when threshed.

Three new methods of treating barley seed against loose smut have been developed during the past 5 years. They are the Spergon and water-soak treatments developed jointly by Russell and Tyner (5, 6, 7, 8, 11, 12, 13), and the anaerobic-incubation treatment devised by Hebert (3). There are several variations of each of the three treatments.

These new treatments represent a real advance in disinfection of barley seed, as a considerable amount of seed can now be treated without much effort. All of them, however, like the hot-water treatment, cause some injury to the treated seed. Tyner and Russell suggested (8, 12) that innate differences, such as maturity of the seed, permeability of the seed coats, and physical injury to seed coats and hull are the main factors affecting the amount of injury from treatment. Arny and Leben (1) noted that seed from some localities tended to be more susceptible to injury than seed from other localities, and attributed the variation to a difference in environmental conditions.

MATERIALS AND METHODS

The experimental work in connection with this study was done during the period 1951-1955. It consisted of field, greenhouse and laboratory tests carried out at Winnipeg whenever new variations of the water-soak

[1] Contribution No. 1549 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Senior Plant Pathologist and Associate Plant Pathologist, respectively, Plant Pathology Laboratory, Winnipeg, Manitoba.

and Spergon treatments appeared. The sowings of seed in the field were in quadruplicate on summerfallow land, with the rows 12 feet long and 9 inches apart. The sowings in the greenhouse were in duplicate, in large beds of unsterilized soil, and the rows of 100 seeds each were 3 feet long and 2 inches apart. The sowings in the laboratory were in quadruplicate, on moistened No. 3 filter paper in Petri dishes, with 25 seeds to a dish.

The barley seed used for this study was obtained mostly from members of the Canadian Seed Growers' Association and was, therefore, of high varietal purity and generally of high germinability. Most of the seed was produced in Manitoba, but some was produced in Quebec, Ontario, Saskatchewan and Alberta.

The seed treatments compared fell into five main groups: A, untreated seed; B, seed treated with Ceresan M (7.2 per cent ethyl mercury p-toluene sulphonanilide); C, seed treated by the water-soak method; D, seed treated with an 0.2 per cent suspension of Spergon (98 per cent tetrachloro-p-benzoquinone); and E, seed treated by the anaerobic-incubation method. As the Spergon and water-soak treatments during the period of this investigation were still being modified, the procedure of treatment was changed from time to time. The full list of seed treatments is as follows:

A. Untreated seed, sown dry.
B. Seed treated with Ceresan M dust at the rate of one-half ounce per bushel, and sown dry.
C. Seed soaked in tap water (70° F.) for 58 hours, and sown as soon as the seed surface became dry.
C1. As in C, but the soaked seed was dried for 3 days before sowing.
C2. As in C1, but the dried seed was treated with Ceresan M before sowing.
C3. Seed soaked for 75 hours in tap water (70° F.), then dried for a week.
C4. As in C3, but soaked only 64 hours.
D. Seed soaked at 70° F. in tap water for 10 hours, then in 0.2 per cent Spergon suspension (also at 70° F.) for another 48 hours, and sown as soon as the seed surface became dry.
D1. Same as D, but the seed was dried for 3 days.
D2. Same as D1, but the dried seed was treated with Ceresan M before sowing.
D3. Seed soaked in 0.2 per cent Spergon suspension for 48 hours at 75° F., then dried for a week.
D4. As in D3, but the temperature was 70° F.
E. Seed soaked for 2 hours, then incubated, under anaerobic conditions at 70° F., for an additional 60 hours, and dried for a week.

For most of the tests, the seed was treated in units of 200 cc. each. This unit was a convenient one as it was small enough to allow the treatment of a number of units simultaneously. At first, the containers used in treating the seed were ordinary 1-quart fruit jars, but later it was found that bags of thin polyethylene film were suitable, as they were light, strong, waterproof and resistant to chemical action. The bags were of various sizes, but a 4-in. × 6-in. size was found the most convenient.

EXPERIMENTAL RESULTS

Preliminary Survey

At the beginning of this investigation very little was known concerning the effect of the new treatments used to control loose smut on the germination of barley seed of different varieties, or of different lots of seed of the same variety. Therefore, a greenhouse experiment was set

TABLE 1.—VARIATION IN PERCENTAGE GERMINATION AMONG DIFFERENT LOTS OF TREATED AND UNTREATED BARLEY SEED

Variety	Seed lot no.	Treatment								Means for seed lots	Means for varieties
		Control	Ceresan M	Water-soak			Spergon				
		A	B	C	C1	C2	D	D1	D2		
Olli	1	70	75	47	51	50	21	33	17	45.5	
	2	100	99	87	92	87	61	55	54	79.4	
	3	70	74	61	65	58	29	47	42	55.7	68.2
	4	99	100	95	96	91	84	73	61	87.4	
	5	98	94	87	88	92	45	33	47	73.0	
Montcalm	6	89	95	91	88	82	38	27	31	67.6	
	7	93	97	60	81	72	17	23	20	55.3	
	8	94	83	74	89	84	48	51	48	72.6	69.5
	9	96	94	92	95	94	77	74	67	86.1	
	10	67	79	79	74	69	52	67	50	67.1	
O.A.C. 21	11	99	96	81	97	94	67	88	87	88.6	
	12	97	97	35	96	98	25	91	90	79.6	
	13	97	100	39	93	93	31	77	80	76.2	81.2
	14	95	95	65	91	84	35	84	63	76.5	
	15	96	97	86	99	91	72	77	70	86.0	
Means for treatments		90.6	91.0	71.9	86.3	82.6	46.8	60.0	55.0		
Significant difference (5%)[1]					10.2					13.9	5.3

[1] Applies, *left to right*, to means for treatments, means for seed lots, means for varieties.

up to provide some information on the scope of the problem and to indicate the direction of future experimentation. It was designed to show the effect of 8 treatments on the germination of 15 lots of barley seed, 5 lots of each 3 varieties, and the results are summarized in Table 1.

The information obtained indicated that, in barley, the seed of different varieties, as well as the seed of different lots of the same variety, varied considerably in response to different treatments. Some lots of seed were much more susceptible to injury than others, and the Spergon treatment was clearly more injurious than the water-soak treatment. There was also an indication (compare treatments C with C1, and D with D1) that the Spergon and water-soak treatments both induced a temporary dormancy in the treated seed, and that a 3-day period of drying restored some of its germinability. After-treatment of the dried seed with Ceresan M had an adverse effect on its germination.

Effect of Different Treatments on the Percentage of Seeds Germinating

In all, five variations of the water-soak treatment (C, C1, C2, C3, C4) and five variations of the Spergon treatment (D, D1, D2, D3, D4) were tested, although not at the same time. The first three variations in each group were tested under greenhouse conditions only, but the remaining two variations of each treatment, as well as one variation of Hebert's anaerobic-incubation treatment, were tested under both greenhouse and field conditions. In each of the greenhouse and field tests, dry untreated seed and dry seed treated with Ceresan M was sown for purposes of comparison.

The results from all these tests are summarized in Table 2. It is shown that the average percentage germination of barley seed treated

TABLE 2.—EFFECT OF DIFFERENT TREATMENTS ON THE PERCENTAGE GERMINATION OF BARLEY SEED

Treatment		Greenhouse test number[1]			Field test number[2]	
		I	II	III	I	II
Check	(A)	90.6	94.0	80.6	82.2	85.4
Ceresan M	(B)	91.0	96.1	88.4	86.3	87.6
Water-soak	(C)	71.9	—	—	—	—
Water-soak	(C1)	86.3	—	—	—	—
Water-soak	(C2)	82.6	—	—	—	—
Water-soak	(C3)	—	—	58.6	—	69.2
Water-soak	(C4)	—	83.5	—	76.4	—
Spergon	(D)	46.8	—	—	—	—
Spergon	(D1)	60.0	—	—	—	—
Spergon	(D2)	55.0	—	—	—	—
Spergon	(D3)	—	—	22.8	—	30.6
Spergon	(D4)	—	57.7	—	67.9	—
Anaerobic incubation	(E)	—	—	85.1	—	82.0
Significant difference (5%)		10.2	6.9	9.3	15.3	11.1

[1] Means for 15 lots of seed in Test I, 26 lots in Test II, and 11 lots in Test III.
[2] Means for 26 lots in Test I, and 11 lots in Test II.

with Ceresan M tended to be higher than it was for untreated seed, and that for seed given the water-soak treatment it was lower. Barley seed treated by any of the variations of the Spergon treatment germinated poorly, while seed treated by the anaerobic-incubation method appeared to be undamaged.

Varietal Differences in Susceptibility to Injury from Seed Treatment

Russell and Tyner (8, 12, 13) mentioned varieties in connection with their discussion of injury from treatment, but, because the samples of each variety they studied were very few, no definite conclusion on this point can be drawn from their work. The results of an exploratory experiment (Table 1) lend some support to the hypothesis that barley varieties differ in their susceptibility to injury from seed treatment and suggest that the variety O.A.C. 21 is less subject to injury than the varieties Olli and Montcalm, but again the paucity of seed samples (5 for each variety) prevents any definite conclusion. However, further support for the hypothesis is provided by data from a recent survey conducted by Arny and Leben (1). They treated seed of seven varieties of barley (5 to 27 samples of each variety) by the water-soak method, and found that the variety Kindred was very susceptible to injury while the variety Wisconsin Barbless was resistant. The varieties Manchuria, Mars, Montcalm, Moore and Oderbrucker were intermediate.

Intra-varietal Differences in Susceptibility to Injury from Seed Treatment

Twenty-six lots of Montcalm barley were collected from seed growers in Quebec, Ontario, Manitoba and Saskatchewan. After each lot was divided into four parts, one part was sown untreated; another part was treated with Ceresan M; the third part was given a water-soak treatment (C4), and the fourth part was given a Spergon treatment (D4). The seed was sown in both the greenhouse and the field, and the plants in the greenhouse were counted when in the 2-leaf stage while those in the field were counted when about to head.

On the average, the germination of seed treated with Ceresan M was higher than that of untreated seed while the germination of seed treated by either the water-soak or the Spergon method was lower, especially when it was treated with Spergon (Table 3). This trend was observed in both the greenhouse and the field. There was a difference, however, in the response of the different lots of seed. Some variation was observed in the germination of seed treated with Ceresan M but for the water-soak and Spergon treatments the variation was of considerable magnitude. As a rule, the seed damaged by the water-soak treatment was damaged more severely by the Spergon treatment. Visually, "susceptible" lots of seed could not be distinguished from "resistant" lots.

It has already been mentioned that some investigators attributed the low germination encountered in seed treated by the hot-water method to a predisposing mechanical injury sustained by the seed in threshing. As mechanically-injured seed generally benefits from treatment with seed protectants, it was thought that the grain in germination of barley seed treated with Ceresan M might be related to the loss in germination when the seed was treated by the water-soak or the Spergon method. Accord-

TABLE 3.—VARIATION IN GERMINATION AMONG DIFFERENT LOTS OF MONTCALM BARLEY SEED TREATED AGAINST LOOSE SMUT

Seed lot no.	Field germination (%)					Greenhouse germination (%)				
	Check (A)	Ceresan M (B)	Water-soak (C4)	Spergon (D4)	Means for seed lots	Check (A)	Ceresan M (B)	Water-soak (C4)	Spergon (D4)	Means for seed lots
1	87	92	83	86	87.0	100	98	95	86	94.7
2	72	89	77	85	80.7	95	97	90	75	89.2
3	80	81	55	29	61.2	93	90	54	15	63.0
4	79	97	70	71	79.2	87	92	69	67	78.7
5	89	86	73	82	82.5	92	96	87	60	86.2
6	67	82	74	75	74.5	93	96	76	66	82.7
7	92	96	85	92	91.2	100	95	94	80	92.2
8	83	87	86	74	82.5	90	96	91	77	88.5
9	84	72	91	72	79.7	99	100	82	40	80.2
10	90	89	87	80	86.5	82	94	86	64	81.5
11	76	92	79	48	73.7	96	99	97	38	82.5
12	52	80	72	51	63.7	93	94	86	21	73.5
13	88	88	86	65	81.7	93	99	92	62	86.5
14	87	90	71	42	72.5	94	98	59	34	71.2
15	82	87	75	73	79.2	89	93	78	89	87.2
16	85	83	83	82	83.2	99	100	95	69	90.7
17	87	81	65	24	64.2	97	94	82	9	70.5
18	84	80	79	78	80.2	93	99	93	78	90.7
19	77	84	82	77	80.0	100	100	98	67	91.2
20	83	83	77	76	79.7	88	99	93	79	89.7
21	85	88	77	87	84.3	91	90	82	89	88.0
22	90	88	62	70	77.5	98	96	60	35	72.2
23	88	81	89	86	86.0	98	96	94	72	90.0
24	89	85	86	76	84.0	92	93	86	62	83.2
25	83	92	59	37	67.7	97	96	67	21	70.2
26	77	88	64	47	69.0	96	97	82	47	80.5
Means for treatments	82.1	86.1	76.4	67.8		94.0	96.1	83.5	57.7	
Sign. diff. (5%)[1]	5.0[1]			14.4[2]		6.9[1]			17.7[2]	

[1]Applies to means for treatments.
[2]Applies to means for seed lots.

TABLE 4.—INTRA-VARIETAL VARIATION WITHIN MONTCALM BARLEY IN ITS RESPONSE TO SEED TREATMENT

Seed lot no.	Percentage deviation from check							
	Greenhouse				Field			
	Check (A)	Ceresan M (B)	Water-soak (C4)	Spergon (D4)	Check (A)	Ceresan M (B)	Water-soak (C4)	Spergon (D4)
1	100	− 2	− 5	−13	87	+ 6	− 4	− 1
2	95	+ 2	− 5	−11	72	+24	+ 7	+18
3	93	− 3	−46	−83	80	+ 2	−31	−64
4	87	+ 6	−21	−24	79	+28	−11	−10
5	92	+ 5	− 6	−35	89	− 3	−18	− 7
6	93	+ 4	−18	−29	67	+23	+10	+12
7	100	− 5	− 6	−20	92	+ 4	− 7	0
8	90	− 7	+ 1	−14	83	+ 5	+ 4	−11
9	99	+ 1	−18	−40	84	−15	+ 8	−15
10	82	+15	+ 4	−22	90	− 1	− 4	−11
11	96	+ 3	−18	−60	76	+21	+ 4	−36
12	93	+ 1	− 8	−77	52	+59	+38	− 2
13	93	+ 4	− 1	−33	88	0	− 2	−26
14	94	+ 4	−37	−64	87	+ 3	−18	−61
15	89	+ 5	−13	0	82	+ 6	− 8	−11
16	99	+ 1	− 4	−31	85	− 2	− 2	− 3
17	97	− 4	−16	−91	87	− 7	−25	−76
18	93	+ 6	0	−16	84	− 5	− 6	− 7
19	100	0	− 2	−33	77	+ 9	+ 7	0
20	88	+12	+ 5	−11	83	0	− 8	− 9
21	91	− 1	−10	− 3	85	+ 4	−10	+ 2
22	98	− 2	−39	−64	90	− 2	−31	−22
23	98	− 2	− 4	−26	88	− 8	+ 1	− 2
24	92	+ 1	− 7	−32	89	− 5	− 4	−15
25	97	− 1	−31	−78	83	+10	−29	−55
26	96	+ 1	−14	−51	77	+14	−17	−26

Coefficients of correlation

Greenhouse data: Between treatments B and C4: +0.4617
Between treatments B and D4: +0.4059
Between treatments C4 and D4: +0.6644

Field data: Between treatments B and C4: +0.5771
Between treatments B and D4: +0.2421 (not significant)
Between treatments C4 and D4: +0.6696

ingly, the values shown in Table 3 were converted into another set of values (Table 4) which show for each seed lot tested the percentage gain or loss in germination from treatment. It was believed that the correlation of the values shown in Table 3 would be somewhat misleading as they were based on the number of seeds sown while those in Table 4 were based on the percentage germination for the untreated seed. In any event, while a correlation of the values in Table 3 showed a significant, positive correlation between the water-soak and Spergon treatments (C4 and D4), but not in any other comparisons, a correlation of the values in Table 4 showed a significant correlation for five of six comparisons. An inspection of the latter coefficients of correlation and of the values in Table 4 reveals that the reaction of Montcalm barley seed to the water-soak and Spergon treatments is negative, with germination decreased, while that to Ceresan M (at the dosage used) is positive, with germination increased. Of course, an overdose of Ceresan M would also reduce germination, especially of mechanically-injured seed.

Effect of Storage on Treated Seed

In a preliminary experiment, the results of which are summarized in Table 1, it was found that when barley seed was treated by the water-soak or the Spergon method and was sown while still wet (treatments C and D), its germination in soil tended to be low. On the other hand, when the seed was dried for 3 days at 70° F. (treatments C1 and D1) before sowing,

TABLE 5.—EFFECT OF STORAGE ON THE PERCENTAGE GERMINATION OF TREATED BARLEY SEED

Seed lot no.	Treatments and days in storage															Significant difference (5%)[1]
	Check (A)			Ceresan (B)			Water-soak (C3)			Spergon (D3)			Anaerobic-incubation (E)			
	0	31	115	0	31	115	0	31	115	0	31	115	0	31	115	
1	92	93	92	96	96	92	72	55	37	16	19	21	98	80	91	10.1
2	84	84	86	94	95	82	63	54	39	45	38	43	83	82	75	
3	91	86	89	84	93	94	90	80	62	33	40	34	95	77	84	
4	91	92	93	84	96	95	75	65	42	24	20	20	91	88	85	
5	87	87	80	92	93	87	58	47	32	29	35	28	82	90	72	
6	61	63	53	72	64	58	7	6	8	15	10	10	62	56	53	
7	97	93	89	95	93	91	66	68	45	22	23	22	88	69	72	
8	84	82	80	90	86	81	18	17	8	6	10	9	76	74	49	
9	96	96	94	95	92	96	93	84	76	45	38	35	93	90	95	
10	78	81	59	72	80	75	37	22	27	7	6	12	73	72	69	
11	88	91	80	95	91	83	63	52	31	8	11	11	93	82	67	
Means for storage periods	86	86	82	88	89	85	59	50	37	23	23	22	85	78	74	4.5
Means for treatments	85			87			49			23			79			7.4

[1] Top to bottom: Applies to means for replicates, means for storage periods, means for treatments.

it germinated much better. There was considerable variation in the response of different lots of seed, even when they were of the same variety. Some were little affected; others responded very noticeably. The original germinability of the seed seemed to bear no relation to its subsequent response to treatment.

In another experiment the treated seed was dried for a week before it was put into paper bags and stored at 70° F. for 115 days. Tests of germinability were made at the beginning of storage and again after 31 and 115 days. As Table 5 shows, untreated seed and that treated with Ceresan M or by the Spergon method did not decrease in germination during storage, but seed treated by the water-soak or the anaerobic-incubation method lost some of its germinability, more so in the former treatment than in the latter. Furthermore, seed from different lots varied even in this respect. In all lots except No. 6, seed given treatment C3 germinated less at the end of the storage period than at its beginning. In seed given treatment E, only about half of the seed lots showed such deterioration.

Observations on treated barley seed incubated on moist filter paper in Petri dishes

Seed from 11 lots of Montcalm barley was treated with Ceresan M (treatment B) or by the water-soak, Spergon and the anaerobic-incubation methods (treatments C3, D3 and E, respectively). After drying for 10 days, the seed was incubated for 5 days on moist filter paper in Petri dishes. The purpose of the incubation was to observe the superficial microflora that might develop, and any change in seed characteristics.

Observations of seed germination in Petri dishes showed once again that different lots of seed vary considerably in their response to treatment. Untreated seed did not germinate as well as it did in soil, but only 3 of the seed lots germinated less than 80 per cent. Seed treated with Ceresan M produced a high percentage of malformed seedlings in 7 seed lots and a low percentage in 4. Treatment by the water-soak and Spergon methods reduced germination very greatly. In 4 of the seed lots treated by the latter two methods, the germination was less than 10 per cent, and in the others less than 50 per cent. Seed treated by the anaerobic-incubation method germinated relatively well, 6 of the lots germinating better than 50 per cent. Only 1 lot of seed was damaged by the latter treatment but that lot contained a considerable proportion of frosted kernels.

Barley seed treated by the Spergon method tended to darken during incubation. The discoloration was moderate to heavy in all 11 lots of seed. A similar but lighter discoloration developed in one lot treated by the water-soak method and in another lot treated by the anaerobic-incubation method. Elsewhere the colour was normal.

A number of fungi were observed growing on the surface of the incubated seed, but, of the genera recognized, *Alternaria* was by far the most common. In the case of untreated seed, a very heavy growth of fungi of this genus occurred in 5 lots of seed, a heavy growth in 1, a moderate growth in 4, and no growth in 1. *Alternaria* did not occur on seed treated with Ceresan M, and there was only a trace in 2 lots that had been treated with Spergon. The water-soak treatment permitted a heavy growth in 1 lot of seed and a moderate growth in 2. Treatment of the seed by the anaerobic-incubation method reduced the growth of *Alternaria* only slightly or not at all.

DISCUSSION

From the foregoing, it is evident that barley seed varies in its response to the treatments currently used for the control of loose smut. Not only do different lots of seed vary in their response to the same treatment but the same lot of seed varies in its response to different treatments. The variation in response, as measured by the effect on seed germination, is attributed by some workers to differences in varietal characteristics, but the evidence provided by others supports the contention of the present writers that the differences in response to treatment are due mostly to differences in the condition of the seed at the time a test is made. The differences in the germination between seed lots numbered 3 and 21, or between seed lots 15 and 17 (Table 3) after the seed had been treated with Spergon, is of a magnitude not likely to be exceeded by the difference produced by a profound difference in varietal characteristics.

It is interesting to speculate on the causes of injury to seed germination from the various treatments used to control loose smut. In the case of the hot-water treatment, temperature is likely to be the most important factor. Treatment of barley at temperatures above 126° F. (129° F. for wheat) lowers germination. For the water-soak treatment, the duration of the soaking period seems to be the dominant factor. In the case of the Spergon treatment, injury to seed germination seems to be due to the

combined effect of immersion and phytotoxicity. For the anaerobic-incubation treatment, tolerance to anaerobic conditions is probably the important factor. In each case, advantage is taken of the fact that the seed embryo is more resistant to these treatments than the smut fungus that infects it.

If it is true that different lots of barley seed are not affected to the same degree by seed treatment, then there arises the problem of how susceptible seed may be detected. Pending the time when a seed treatment that causes no harm is discovered, the alternative options left to the farmer are as follows: He may buy seed which has been found to be free or nearly free from smut, or may buy seed that has been treated and tested for germination. A third option for him is to treat a small portion of his seed and then test it for germination himself. If the germination is low (below 80 per cent) a proportionate increase in seeding rate is advised. Since visual examination of barley seed is not a sure way of determining the post-treatment germinability, one of the options suggested above will probably have to be exercised.

REFERENCES

1. Arny, D. C., and C. Leben. The effect of the water-soak seed treatment on the germination of certain barley varieties grown at different locations. Phytopathology 45 : 518-519. 1955.
2. Heald, F. D. Manual of plant diseases. 1st ed. McGraw-Hill Book Co., New York, N.Y. 1926.
3. Hebert, T. T. A new method of controlling loose smut of barley. Plant Disease Reptr. 39 : 20-22. 1955.
4. Hurd, Annie May. Seed-coat injury and viability of seeds of wheat and barley as factors in susceptibility to molds and fungicides. J. Agr. Research 21 : 99-122. 1921.
5. Russell, R. C. A study of the hot water treatment of barley for the control of loose smut, *Ustilago nuda*. Sci. Agr. 30 : 303-315. 1950.
6. Russell, R. C. The "drowning treatment" for loose smut of barley. Proc. Can. Phytopath. Soc. (1952), 20 : 21. 1953.
7. Russell, R. C. Progress report on the control of loose smut of barley by the cod water soak treatment. Proc. Can. Phytopath. Soc. (1953), 21 : 16-17. 1951.
8. Russell, R. C., and L. E. Tyner. The influence of temperature on the time required to control loose smut of barley by means of the Spergon or water-soak treatments. Can. J. Agr. Sci. 34 : 533-538. 1954.
9. Schafer, R. A., and E. D. Hansing. Effect of hot-water treatment on emergence of spring barley and control of brown loose smut. Phytopathology 40 : 518-521. 1950.
10. Tapke, V. T. Effect of the modified hot water treatment on germination, growth and yield of wheat. J. Agr. Research 28 : 79-97. 1924.
11. Tyner, L. E. Control of loose smut of barley by chemical and physical treatments. Sci. Agr. 31 : 187-192. 1951.
12. Tyner, L. E., and R. C. Russell. Control of loose smut in barley by tetrachloroparabenzoquinone (Spergon SL). Plant Disease Reptr. 36 : 180-181. 1952.
13. Tyner, L. E. The control of loose smut of barley and wheat by Spergon and by soaking in water at room temperature. Phytopathology 43 : 313-316. 1953.

A COMPARISON OF MCP AND 2,4-D FOR THE CONTROL OF ANNUAL WEEDS IN GRAIN CROPS[1]

H. A. FRIESEN AND D. R. WALKER[2]

[Received for publication June 22, 1956]

ABSTRACT

Oats, flax and barley were sprayed with various formulations of MCP (2-methyl-4-chlorophenoxyacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) at a number of Experimental Farms in Western Canada in 1954 and 1955. Each formulation was applied at 4 and 8 ounces of acid equivalent per acre at each of two dates, viz., *Date 1*, when the weeds were in the seedling stage less than 3 inches in height; and *Date 2*, at a stage coinciding with the first appearance of buds on the major weed species.

Stinkweed (Thlaspi arvense, L.), wild mustard (Brassica kaber (DC.) L.C. Wheeler var. pinnatifida (Stokes) L.C. Wheeler), lamb's quarters (Chenopodium album L.), Russian pigweed (Axyris amarantoides L.), and ball mustard (Neslia paniculata L.), were effectively controlled by each of the MCP and 2,4-D formulations used. Russian thistle (Salsola kali L.), and red-root pigweed (Amaranthus retroflexus L.), were not satisfactorily controlled by MCP, whereas hemp nettle (Galeopsis tetrahit L.) was not controlled by 2,4-D. Wild buckwheat (Polygonum convolvulus L.) was not controlled by any of the treatments. Each treatment was more effective when applied during the seedling stage of the weeds.

On the basis of weed control and yield of grain the results of this study favour the use of MCP over 2,4-D with either oats or flax. Oats was significantly more tolerant to MCP, particularly if treated during the early growth stages when weed competition was most critical. With flax, the difference in tolerance was most pronounced in favour of MCP at the later date of treatment. The 2,4-D ester and low volatile ester resulted in a preponderance of plant deformities, lowered the yields of both oats and flax and delayed the maturity of flax significantly. Barley yields in this study were not adversely affected by any of the treatments.

INTRODUCTION

Selective post-emergence spraying with 2,4-D has been carried out on an estimated 12,000,000 acres of grain crops in each of the past 5 years, in Western Canada. In the past 4 years farmers and research workers have noted an increase in the incidence of crop injury, particularly to oats and flax treated with 2,4-D ester under conditions conducive to rapid crop growth. This situation stimulated interest in the possible use of MCP in place of 2,4-D. The results of preliminary trials with MCP formulations indicated that they gave unsatisfactory control of Russian thistle, one of the major weeds of the spring wheat growing areas. On the other hand, it was observed that hemp nettle was more effectively controlled by MCP than by 2-4-D. The results of these trials indicated that, while the degree of weed control was sometimes less with MCP than it was with 2,4-D, the yield of grain was usually higher on the MCP treated plots. This was especially true where oats or flax was the treated crop. However, these preliminary tests failed to clearly establish whether the reaction of crops and weeds to MCP and 2,4-D was sufficiently different to offset the higher cost of the MCP.

[1] Contribution from the Division of Field Husbandry, Soils and Agricultural Engineering, Experimental Farms Service, Canada Department of Agriculture.

[2] Senior Agronomist and Project Leader, and Research Officer, respectively, Experimental Farm, Lacombe, Alberta.

At a Field Husbandry Divisional Staff Meeting in 1953, a committee was appointed to draw up a comprehensive project to compare MCP and 2,4-D on oats, flax and barley under varying weed conditions in Western Canada. The senior author was named project leader. This paper reports the results of this study.

REVIEW OF LITERATURE

During the 5 years immediately following the introduction of 2,4-D into Western Canada in 1945, weed research was largely devoted to studying the effects of this herbicide on crop and weed species. It was not until 1949 that the MCP formulations were included in this work.

Tandon (17), working with flax, noted that, while varieties tended to show a varying response to treatment with different 2,4-D formulations, all varieties were less affected by the sodium salt than by the amine or ester formulations of 2,4-D.

Olson *et al.* (13) studied the reaction of wheat and barley to 2,4-D and found that these crops were most sensitive to treatment at two widely separated growth periods. The first was an early seedling stage, when the plants were 1 to 5 inches tall while the second extended from the time that the plants were well into boot stage until a few days prior to heading. Comparable results were reported by Friesen (6), Leggett (10) and Friesen and Olson (9). The latter workers, using 2,4-D ester at 1- to 2-day intervals from emergence until after heading, established that with oats the sensitivity is evident at the seedling stage but continued over a much longer period than in barley.

Olson (14), in a summary of the results of comparative tests of 2,4-D and MCP on oats conducted by a number of workers in Western Canada, noted that, with respect to crop damage, the use of MCP was preferable to 2,4-D.

Dunham (5), in summing up the results of coordinated trials with herbicides on flax in the North Central States and Western Canada, reported that even very low dosages of 2,4-D reduced the yield of weed-free flax. The common weeds of flax were relatively more susceptible to 2,4-D than the crop, and MCP was less injurious than 2,4-D. Similar findings were reported by McDonald (12).

Burrows and Olson (1,2) studied the competitive effect of varying levels of wild mustard on the yields of wheat and flax. They showed that the removal of an infestation of 10 mustard plants per square yard by spraying with 2,4-D resulted in higher yields of either crop. Early spraying was found to be essential. They concluded that wheat should be sprayed not later than the 4-leaf stage of growth and flax should be treated during the seedling stage. Shebeski (15) expanded these studies to include the treatment of oats with 2,4-D and MCP at five different growth stages. His results suggested that oats should be sprayed with 2,4-D or MCP not later than the 4-leaf stage of growth to effectively remove weed competition. However, during this period the application of 2,4-D resulted in highly significant yield losses due to crop injury. This was not true when MCP was used in place of 2,4-D.

Differential response of some weed species to 2,4-D and MCP was reported as early as 1949. Friesen (7) observed that Russian thistle was satisfactorily controlled by 2,4-D but not by MCP. Coupland (3) and Sexsmith (16) have each confirmed this finding. Craig (4) noted that lady's thumb (Polygonum persicaria) could be controlled with 2,4-D but not with MCP. Leggett (11) reported that hemp nettle appeared to be tolerant to 2,4-D but was controlled to some extent by MCP. Friesen (8) in a preliminary trial noted that the degree of control obtained by spraying hemp nettle with MCP during the seedling stage was sufficient to result in a significant increase in the yield of oats. However, 2,4-D applied under similar conditions in this test did not control the nettle nor increase the yield of grain.

MATERIALS AND METHODS

This experiment was conducted at each of seven locations during the years 1954 and 1955. The locations were at the Experimental Farms at Lacombe in Alberta; at Melfort, Scott, Swift Current and Regina in Saskatchewan; and at Brandon and Morden in Manitoba. Unless otherwise stated the procedures were identical at all locations in both of the years.

Three formulations each of MCP and 2,4-D were applied at two rates and at two stages of crop growth in a balanced lattice design. The formulations were the commercially formulated sodium salt, triethanolamine and butyl ester of MCP and the amine (mixed), butyl ester and low volatile propylene glycol butyl ether ester of 2,4-D. Rates were 4 and 8 ounces of acid equivalent per acre. The first date of spraying was when the weeds were less than 3 inches in height (seedling stage) and the second date was at the time flower buds first appeared on the major weed species.

All herbicides were supplied directly from the manufacturer to ensure uniformity of product. Water at approximately 5 gallons per acre was used as the diluent in the spray mix. Spraying was done with a small plot sprayer. At each of the locations the natural infestation of weed seeds in the soil was augmented by planting a mixture of the seeds of the annual weed species common to the region. No other attempt was made to equalize the density of the weed infestation prior to the application of the herbicides.

The experimental design, including a check or unsprayed plot as one of the 25 treatments, was replicated six times at each location. Plots were 20 feet long and 8 to 10 feet wide depending on the width of seed drill available at the various locations.

Crops used varied with locations. Lacombe and Morden used oats and flax, respectively, in both years. Melfort used flax and barley; Swift Current, wheat and flax; and Scott, wheat and oats in 1954 and 1955 respectively. Brandon and Regina conducted the test in 1955 using flax as the experimental crop.

The results of the treatments on the weed growth were evaluated by recording weed density before and after spraying. For this purpose an actual weed count was taken at two fixed locations chosen at random in each plot, utilizing 1 square yard of area at each location. The two or three major weed species at each project location were counted separately

while the counts of the minor species were bulked. In order to obtain an assessment of those treatments which did not kill the weeds but did suppress their growth, the weeds on a part of each plot were harvested at heading time of the crop for dry weight determinations. This was accomplished by clipping the weeds at ground level on an area 20 square feet in size on each plot. A species separation similar to that used for the counts was made prior to oven drying.

In 1954, point sampling was attempted as a method of measuring the density of weeds before and after spraying. Five readings with a 36-point frame were made on each plot. The technique was very satisfactory for the before-spraying assessment, when the weeds were small. However, difficulty arose with later readings because of the trampling of the crop and the branching habit of most of the weeds species, which rendered the reading of per cent ground cover virtually meaningless. Consequently, the method was abandoned.

The effect on the crop was measured by visual estimates or ratings of morphological deformities and by the yield of grain. The grain yield was determined by harvesting and threshing two rod rows from each plot.

EXPERIMENTAL RESULTS

Excessive precipitation in the early part of 1954 caused flooded conditions in many areas of Western Canada. Consequently experimental results were reported only from Lacombe, Alberta, and Melfort, Saskatchewan, in that year. In 1955 data were received from each of the co-operating Farms.

Results on Eagle Oats at Lacombe, Alberta

The predominant weed species was stinkweed, which accounted for some 50 per cent of the entire weed population. Hemp nettle was the second major species and accounted for roughly 25 per cent of the population. In order of prevalence, ball mustard, lamb's quarters, Russian pigweed, chickweed, (Stellaria media) and green foxtail (Setaria viridis), constituted the remainder of the population. The treatments, weed densities, dry weights of weeds and yields of oats per acre recorded in 1954 are given in Table 1.

As shown in Table 1, all of the treatments at both dates of application resulted in almost complete removal of the stinkweed. Equally good control of ball mustard, lamb's quarters and Russian pigweed was observed. The low plant counts noted under the column "Others" were made up of chickweed and green foxtail, which were largely unaffected by any of the treatments. The counts of hemp nettle given in Table 1 showed that the MCP formulations applied at *Date 1*, the seedling stage of the weed, resulted in some reduction in the number of plants. The 8-ounce application of MCP was more effective than the 4-ounce rate. Less response was noted following treatment with each MCP formulation at *Date 2*, when the nettle was in the early bud stage. The 2,4-D formulations did not measurably affect the number of hemp nettle plants at either date of application.

A more accurate assessment of the hemp nettle control may be obtained from the dry weights of weeds given in the table. These dry weights,

with the exception of the check, were almost entirely hemp nettle. The suppression of nettle growth caused by the MCP formulations at *Date 1* was very striking. On the other hand, the 2,4-D formulations had virtually no effect on the growth or development of the nettle as shown by the high dry weights recorded. At the early bud stage, *Date 2*, the MCP formulations were notably less effective in suppressing the nettle growth than when applied at *Date* 1 when both the number of plants and the dry weights were considered. The 2,4-D formulations again had little or no effect on this weed. Variability in the density of the nettle population over the plot area was in evidence in the data presented but was not sufficient to mask the trends of effects on the weeds and the crop of the various formulations.

The effect of the control of hemp nettle as the result of spraying at the seedling stage, *Date 1*, was reflected in the yield of oats. As shown in Table 1, each of the MCP treated plots exceeded the yield of the untreated check plot. With the exception of MCP amine at 4 ounces, and the sodium salt at 4 and 8 ounces, the differences in yield were statistically significant. The yield increases following these treatments were just

TABLE 1.—NUMBER AND WEIGHT OF WEEDS AND YIELD OF OATS FOLLOWING TREATMENT WITH MCP AND 2,4-D AT LACOMBE, 1954.

Treatment	Rate oz./ acre	Date	Weed count per sq. yard			Dry wt. of weeds in lb./acre		Yield bu./ac. of oats
			H.N.[1]	S.W.	Others	H.N.	Others	
Check	0	0	64	164	82	457	427	35.0
MCP ester	4	1	76	1	3	173	0	42.2*
MCP ester	8	1	20	0	3	33	0	43.0*
MCP sod. salt	4	1	25	1	11	32	13	38.7
MCP sod. salt	8	1	18	1	7	27	3	38.5
MCP amine	4	1	17	0	9	21	6	40.2
MCP amine	8	1	5	1	3	4	2	43.2*
Average			27	1	6	48	4	41.0
2,4-D ester	4	1	142	3	6	549	0	33.1
2,4-D ester	8	1	121	0	4	719	0	36.9
2,4-D amine	4	1	57	8	9	427	16	38.7
2,4-D amine	8	1	35	0	3	133	3	40.7
2,4-D low volatile	4	1	111	1	1	645	0	37.0
2,4-D low volatile	8	1	80	0	4	393	4	33.1
Average			91	2	4	478	4	36.6
MCP ester	4	2	62	0	12	292	4	35.0
MCP ester	8	2	41	0	1	149	0	33.0
MCP sod. salt	4	2	28	0	6	53	2	40.1
MCP sod. salt	8	2	92	0	2	292	1	38.2
MCP amine	4	2	19	0	5	28	4	41.4
MCP amine	8	2	25	0	2	57	0	37.5
Average			44	0	5	145	2	37.5
2,4-D ester	4	2	127	0	4	420	0	28.6
2,4-D ester	8	2	23	0	2	81	0	22.4*
2,4-D amine	4	2	54	0	6	249	2	36.0
2,4-D amine	8	2	57	0	1	325	0	29.7
2,4-D low volatile	4	2	49	0	2	331	0	28.6
2,4-D low volatile	8	2	73	0	1	464	0	23.3*
Average			64	0	3	312	0.3	28.1

* Difference from the check plot greater than the L.S.D. of 6.4 bushels per acre for the 0.05 level of probability.

[1] H.N.—Hemp Nettle
S.W.—Stink Weed

below the necessary difference of 6.4 bushels per acre required for statistical significance. The 2,4-D ester and low volatile ester did not control the nettle and resulted in little or no increase in the yield of oats, notwithstanding that each of the treatments had removed the heavy infestation of stinkweed and most of the "other" weeds also present on these plots. The preponderance of plant deformities, especially following the 8-ounce per acre dosage of each of the 2,4-D esters, would suggest that the failure of the 2,4-D ester treatments to increase the yield was partly due to herbicidal injury to the oats. The yields following treatment with the 2,4-D amine were higher but not significantly so.

At *Date 1*, the oat crop was in the 4-leaf stage of growth at the time of treatment. Plant deformities were not in evidence following treatment with any of the MCP formulations. A few leaf abnormalties were noted following treatment with the 2,4-D amine. Tubular leaves, deformed heads, enlarged nodes and thickened and stunted stems were noted on nearly all plants on the plots treated with the 2,4-D ester and low volatile ester (see Figures 1 to 3 inclusive). The abnormalities were more severe where the 8-ounce per acre rate of application of these formulations was used.

At *Date 2*, none of the treatments resulted in significant yield increases. Furthermore, the crop being in the jointing stage was more susceptible to herbicide injury, as evidenced by the depressing effect on the yield following the 2,4-D treatments. As shown in Table 1, the 2,4-D ester and low volatile ester at 8 ounces per acre significantly depressed the yield of oats, despite the removal of the heavy infestation of stinkweed, ball mustard and lamb's quarters.

The treatments, weed densities, dry weights of weeds and yields of oats per acre recorded at Lacombe in 1955 are given in Table 2. Also given in this table are the yields of oats from this test at Scott, Saskatchewan. The weed species encountered at Lacombe in 1955 were similar to those reported in 1954, but hemp nettle rather than stinkweed was the predominant species. In general, it might be stated that with some variations the control of the weeds and the yields of oats followed much the same pattern as that recorded in the previous year. In 1955, weather conditions were conducive to very rapid growth, which probably accounted for the much higher incidence of morphological abnormalities and depressed yields of oats than was noted in 1954. Thus, yield reductions were observed following the application of all the 2,4-D treatments, notwithstanding that each treatment resulted in the removal of at least one-half of the weed infestation.

The hemp nettle infestation in 1955 was extremely dense. As in 1954, the 2,4-D treatments had no measurable effect on this weed. The MCP formulations at 8 ounces per acre killed up to 30 per cent of the plants and suppressed the growth of the survivors. As shown in Table 2, the MCP formulations at 4 ounces per acre were decidedly less effective on this weed species. Again, as in 1954, the control of all weed species was more effective if they were treated during the seedling stage, *Date 1*. A salient observation was that MCP, unlike the 2,4-D treatments, did not significantly reduce the yield of oats at either date of application. Indeed, the MCP treatments, notably at *Date 1*, increased the yields to some extent.

FIGURE 1. Tubular leaves on oats (*right*) due to spraying with 2,4-D low volatile ester at 8 oz. per acre during the 4-leaf stage. This treatment did not control the heavy nettle infestation. Plot on *left* not sprayed.

FIGURE 2. Tubular leaves on oats (*centre plot*) due to spraying with 2,4-D ester at 4 oz. per acre during the 4-leaf stage.

FIGURE 3.—Abnormal oat plants in plot *in foreground* due to spraying 2,4-D low volatile ester during the 4-leaf stage.

TABLE 2.—NUMBER AND WEIGHT OF WEEDS AND YIELD OF OATS FOLLOWING TREATMENTS WITH MCP AND 2,4-D FORMULATIONS AT LACOMBE, ALBERTA, AND SCOTT, SASKATCHEWAN IN 1955

Treatment	Rate oz./ acre	Date	Weed count per sq. yard			Dry wt. of weeds in lb./acre		Oat yield bu./ac.	
			H.N.	S.W.	Others	H.N.	Others	Lacombe	Scott
Check	0	0	217	12	36	400	740	59.0	79.4
MCP ester	4	1	156	2	15	260	72	68.2	87.1*
MCP ester	8	1	233	0	7	148	32	59.8	86.1*
MCP sod. salt	4	1	171	8	28	404	88	62.0	86.1*
MCP sod. salt	8	1	97	8	18	204	28	60.2	79.3
MCP amine	4	1	253	8	20	420	68	58.1	86.7*
MCP amine	8	1	220	7	12	232	88	67.1	88.4*
Average			188	6	17	278	63	62.6	85.6
2,4-D ester	4	1	324	0	9	442	0	51.8	64.5*
2,4-D ester	8	1	143	1	4	348	0	46.5	58.1*
2,4-D amine	4	1	175	15	24	416	64	58.2	79.4
2,4-D amine	8	1	259	2	12	548	16	46.0	77.0
2,4-D low volatile	4	1	300	0	8	432	12	37.0*	66.5*
2,4-D low volatile	8	1	220	0	6	700	18	35.7*	72.9*
Average			237	3	11	481	18	45.9	69.7
MCP ester	4	2	129	4	17	444	84	63.2	79.6
MCP ester	8	2	61	1	11	264	176	56.0	73.3*
MCP sod. salt	4	2	99	10	25	252	112	58.0	88.4*
MCP sod. salt	8	2	29	1	14	128	88	71.2	85.7*
MCP amine	4	2	441	0	13	248	72	46.1	77.6
MCP amine	8	2	34	1	10	300	28	66.5	83.4*
Average			132	3	15	273	93	60.2	81.3
2,4-D ester	4	2	141	3	12	340	44	48.5	84.9*
2,4-D ester	8	2	261	3	5	452	36	35.5*	74.9*
2,4-D amine	4	2	250	1	36	380	204	42.4*	78.2
2,4-D amine	8	2	242	1	9	456	0	41.2*	86.3*
2,4-D low volatile	4	2	161	0	8	412	72	37.9*	79.8
2,4-D low volatile	8	2	173	0	6	336	68	36.8*	72.2
Average			205	1	13	397	71	40.4	79.4

* Differences from control greater than L.S.D. of 13.3 bushels per acre at Lacombe or 3.1 bushels per acre at Scott for the 0.05 level of publicity.
H.N.—Hemp Nettle
S.W.—Stink Weed

1955 Results with Exeter Oats at Scott

In this trial, wild buckwheat was the major weed species on the plot area. Just prior to applying the herbicides the buckwheat seedlings were eaten by a leaf feeding beetle, (Gastrophysa polygoni L.). Since the oats were not harmed by the beetles the yield data presented in Table 2 illustrate the effect the formulations had on oats under nearly weed-free conditions. As shown in the table the oat yields were not significantly decreased by any one of the MCP treatments at either date of application, with the exception of the MCP ester at 8 ounces at *Date 2*. The 2,4-D ester formulations significantly reduced the oat yields, at *Date 1*, when the crop was in full 5-leaf stage. At *Date 2*, when the oats were in the early boot stage the 2,4-D ester treatments at 8 ounces reduced the yield.

1954 Results with Flax at Melfort, Saskatchewan

As shown in Table 3, a severe infestation of weeds was encountered on the Melfort plots. The species in descending order of prevalence were

TABLE 3.—NUMBER AND PER CENT CONTROL OF WEEDS AND YIELD OF FLAX FOLLOWING TREATMENT WITH MCP AND 2,4-D AT MELFORT, 1954

Treatment	Rate oz./acre	Date	Weeds other than Wild Buckwheat		Yield of flax bu./acre
			Init. sq. yd. counts	% control	
Check	0	0	32	−99.1	9.8
MCP ester	4	1	26	82.3	17.2*
MCP ester	8	1	28	100.0	20.0*
MCP sod. salt	4	1	34	89.5	14.1*
MCP sod. salt	8	1	29	92.0	15.2*
MCP amine	4	1	22	61.8	17.7*
MCP amine	8	1	30	78.6	17.4*
Average			28	84.0	16.9
2,4-D ester	4	1	32	98.1	13.6*
2,4-D ester	8	1	22	95.6	10.0
2,4-D amine	4	1	26	72.6	13.0*
2,4-D amine	8	1	27	86.8	11.7
2,4-D low volatile	4	1	27	94.1	9.5
2,4-D low volatile	8	1	19	94.7	8.4
Average			26	90.3	11.0
MCP ester	4	2	34	55.9	13.4*
MCP ester	8	2	34	81.5	15.9*
MCP sod. salt	4	2	27	10.0	14.2*
MCP sod. salt	8	2	29	45.4	13.7*
MCP amine	4	2	32	−14.4	13.9*
MCP amine	8	2	30	39.0	13.2*
Average			31	36.2	14.1
2,4-D ester	4	2	35	94.3	8.3
2,4-D ester	8	2	30	95.7	7.0
2,4-D amine	4	2	28	3.6	12.3
2,4-D amine	8	2	29	55.2	8.2
2,4-D low volatile	4	2	29	89.8	9.3
2,4-D low volatile	8	2	35	99.1	7.2
Average			31	73.0	8.7

* Difference from the check plot greater than the LSD of 3.2 bu./ac. for the 0.05 level of probability.

wild mustard, wild buckwheat, Russian pigweed, stinkweed, and blue burr (Lappula echinata). Growing conditions were excellent at both dates of treatment. Each of the MCP and 2,4-D ester treatments resulted in upwards of 90 per cent control of each weed species, except wild buckwheat. The MCP and 2,4-D amines and the MCP sodium salt resulted in 60 to 80 per cent control of these weed species. None of the treatments applied in 1954 had any effect on the wild buckwheat. The control of each species was observed to be somewhat less effective at the second date of application of each treatment. The dry weights of weeds were not obtained.

The yields of flax presented in Table 3 show a decided advantage in favour of the MCP formulations. Each MCP treatment, at each rate and date of application resulted in a significant increase in the yield of flax. The 2,4-D treatments, with two exceptions, viz., 2,4-D ester at 4 ounces per acre at *Date 1* and the 2,4-D amine at 4 ounces per acre at *Date 2*, failed to give a significant yield increase. Actually the 2,4-D ester and the low volatile ester depressed the yields but not significantly.

Cool, wet weather in the fall prevented the securing of actual maturity dates. However, the effect of the delayed maturity caused by some of the treatments was well illustrated by a killing frost on September 16, 1954. The untreated or check plots contained 30 per cent frozen seed, the plots treated with the MCP sodium salt and MCP amine at the 4-ounce per acre rate at *Date 1* contained 29 to 40 per cent, and all other MCP treatments contained 40 to 65 per cent frozen seed. Each of the 2,4-D treatments had over 65 per cent of the flax seed frozen. The 8-ounce per acre rate of each 2,4-D formulation applied at *Date 2* had 100 per cent of the seeds frost damaged.

TABLE 4.—WEIGHT OF WEEDS AND YIELD OF FLAX FOLLOWING TREATMENT WITH MCP AND 2,4-D AT REGINA, SWIFT CURRENT AND MORDEN, 1955

Treatment	Rate oz./ac.	Date	Regina, Sask.		Swift Current, Sask.		Morden, Man.	
			Weeds	Flax	Weeds	Flax	Weeds	Flax
Check	0	0	1175	9.6	333	22.5	989	14.8
MCP ester	4	1	610	13.0*	48	22.6	37	19.3*
MCP ester	8	1	860	11.8*	178	24.4	11	16.8
MCP sod. salt	4	1	995	9.3	50	22.1	22	16.2
MCP sod. salt	8	1	555	12.0*	35	21.9	32	15.8
MCP amine	4	1	725	12.2*	175	20.5	74	16.9
MCP amine	8	1	915	13.4*	123	25.5	37	17.4
Average			777	12.0	102	22.8	36	17.1
2,4-D ester	4	1	550	14.5*	190	19.6	33	14.9
2,4-D ester	8	1	540	10.7	13	21.0	9	15.4
2,4-D amine	4	1	740	10.0	378	20.1	34	17.2
2,4-D amine	8	1	730	10.6	50	20.9	16	17.1
2,4-D low volatile	4	1	650	10.1	220	22.7	9	14.2
2,4-D low volatile	8	1	730	10.1	45	21.2	14	15.4
Average			657	11.0	149	20.9	19	15.7
MCP ester	4	2	660	6.8*	93	22.3	781	16.4
MCP ester	8	2	1360	6.9*	260	19.6	801	16.1
MCP sod. salt	4	2	1045	7.2*	360	20.3	741	18.7*
MCP sod. salt	8	2	1315	7.4*	178	24.8	171	14.3
MCP amine	4	2	805	8.6	290	20.0	641	14.9
MCP amine	8	2	730	7.8*	268	22.3	604	15.2
Average			986	7.5	242	21.6	623	15.9
2,4-D ester	4	2	1080	6.4*	400	20.9	622	13.0
2,4-D ester	8	4	685	5.0*	150	18.2	474	8.4*
2,4-D amine	4	2	810	6.9*	157	22.7	642	14.7
2,4-D amine	8	2	865	5.7*	93	19.3	521	12.0
2,4-D low volatile	4	2	675	6.7*	123	18.5*	721	8.9*
2,4-D low volatile	8	2	635	5.4*	98	18.4*	558	10.7
Average			792	6.0	170	19.7	590	11.3
* L.S.D. P — 0.05				1.6		4.0		3.8

NOTE: "Weeds" denotes the oven-dry weight of weeds in lb./acre after spraying. The high weight of weeds at Regina, Sask. was due to a heavy infestation of wild oats.
"Flax" denotes the yield of flax in bushels per acre.

1955 Results with Flax at Regina and Swift Current in Saskatchewan and at Brandon and Morden in Manitoba

The major weed species present at each of the four locations were stinkweed, lamb's quarters, wild mustard and wild buckwheat. Although the control of the three first named weed species was somewhat better with the 2,4-D ester formulations than it was with the 2,4-D amine and each of the MCP formulations, the differences were not of a significant magnitude. In other words each formulation and rate resulted in satisfactory control of each of these species. Small differences in the degree of weed control were noted in favour of spraying at the early stage and the use of the heavier 8-ounce per acre rate of application. At Brandon, it was of particular interest that Russian thistle was effectively controlled by each of the 2,4-D ester and low volatile ester treatments and to a lesser extent by the MCP ester at the 8-ounce per acre rate, but was not killed or controlled by any one of the remaining treatments in this test.

The dry weight of weeds and the yields of flax recorded at three of the four locations are presented in Table 4. At Brandon the results were not reported for individual treatments but were bulked according to formulations. These are presented in Table 5. At each of the four locations the highest yields of flax were realized following treatment with the MCP formulations. Furthermore, the yields were higher when the treatments were applied during the seedling stage (*Date 1*) than when applied during the early bud stage of the weeds (*Date 2*). The yield differences between the MCP and 2,4-D treatments at Regina, Morden, and Swift Current were not large but consistently favoured the MCP formulations. At Brandon the yields of flax following the use of the MCP formulations and the 2,4-D amine formulation were decidedly larger than where either the 2,4-D ester or the low volatile ester was used.

TABLE 5.—WEED CONTROL EXPRESSED IN PER CENT OF THE CHECK AND YIELD OF FLAX FOLLOWING TREATMENT WITH MCP AND 2,4-D AT BRANDON, 1955

Formulation	Flax yield bu./ac.	Weed control %
Check	23.1*	0
2,4-D ester	23.4	88.5
2,4-D amine	26.9	65.0
2,4-D low volatile	23.6	91.2
MCP ester	27.2	87.8
MCP sod. salt	26.3	77.0
MCP amine	26.4	77.2
Average date 1	25.3	78.9
Average date 2	25.1	82.5
Average 4 oz./ac.	25.8	75.4
Average 8 oz./ac.	25.5	85.5

* Each statistic represents an average of the results obtained following the application of the respective formulations at two rates and two dates of spraying.

The differential effect of the formulations on the date of maturity of the flax favoured the MCP at each location in 1955. The untreated flax matured 1 to 2 days earlier than the plots treated with MCP. The 2,4-D ester and low volatile ester treated flax required 4 to 11 more days to mature than did the MCP treated flax.

1955 Results with Barley at Melfort, Saskatchewan

The weed infestation was very light and consisted chiefly of stinkweed and wild mustard. The barley was 6 to 8 inches tall at the first date of spraying and in the late shot blade stage at the second date of spraying. Weed control was good at both dates but the kills were decidedly more rapid and extensive at the early date of spraying.

Yield differences between treatments were small and not statistically significant. Deformities in the barley were rare and maturity was normal following all treatments. Since no significant differences in either control of weeds or yield of crop were recorded the actual data have not been presented.

DISCUSSION

The experimental evidence procured in this study has shown that stinkweed, wild mustard, lamb's quarters, Russian pigweed and ball mustard were controlled quite effectively with each of the MCP and 2,4-D formulations used. While the 2,4-D ester and low volatile ester and the MCP ester were more rapid and somewhat more positive in their action on these weeds than the other formulations, the differences were not significant. Wild buckwheat was not controlled by any of the treatments applied in these tests. Russian thistle and red-root pigweed were controlled effectively with the 2,4-D ester and low volatile ester whereas the remaining treatments did not give satisfactory control of these two species. On the other hand, hemp nettle was affected little by any of the 2,4-D treatments. Each of the MCP treatments resulted in a marked suppression of growth of this species.

At each of the locations, except Brandon in 1955, the herbicide treatments were more effective if applied during the seedling stage rather than at the bud stage of the weeds. The 8-ounce rate generally was more effective than the 4-ounce rate of application. The differences in effectiveness between rates of application of a given formulation were not of a significant magnitude except where the more resistant species such as red-root pigweed, Russian thistle and hemp nettle were encountered.

The effect of the various herbicide treatments on the yields of oats and flax favoured the use of MCP over 2,4-D. Oats was observed to be decidedly more tolerant to MCP at the early growth stages; this usually coincides with the time at which weed competition is most critical. In this regard the study supported the findings of Burrows and Olson (1, 2) and Shebeski (15) who have pointed out the urgency for the early removal of weeds with herbicides if undue competition with the crop is to be averted. At Lacombe, in 1954 and 1955, the oat plots were heavily infested with stinkweed and hemp nettle. Each of the treatments resulted in nearly complete elimination of the stinkweed. The MCP formulations at the

8-ounce and to a lesser extent at the 4-ounce rate applied during the seedling stage, killed some of the nettle and suppressed the growth of the survivors, which in turn increased the yield of the oats. The 2,4-D treatments made at this stage of growth not only failed to suppress the nettle but injured the oats. Thus, in 1954 the removal of the stinkweed competition by the 2,4-D was not reflected in higher yields. In 1955, each of the 2,4-D formulations depressed the yields. This discrepancy in crop yields has been ascribed to the excellent growth conditions in 1955 which were more conducive to herbicidal injury to the crop, rather than to the somewhat heavier nettle infestation. The significant yield decreases following the use of the 2,4-D ester and low volatile ester on nearly weed-free oats at Scott support this theory.

At Lacombe the late stage of treatment, *Date 2*, permitted the more prolonged competition from both the stinkweed and the hemp nettle, which resulted in the failure of any of the MCP treatments to significantly increase the yield. The *Date* 2 spraying was done as soon as the first buds appeared on the stinkweed, the major weed species. At this time the oats had not yet reached the boot or more tolerant stage, hence, crop injury and reduced yields were noted following the application of the 2,4-D formulations. At Scott, where essentially no weed infestation existed on the plots in 1955, the spraying was done later, in the early boot stage and yield reductions were noted only where the 2,4-D ester and low volatile ester were applied at the rate of 8 ounces per acre.

The effect of this greater tolerance on the yield of oats was sufficient to offset the higher cost of the MCP by a wide margin. Where hemp nettle constitutes a major part of the weed infestation the benefits from the use of MCP rather than 2,4-D were particularly outstanding.

The results with flax at each of the co-operating Experimental Farms favoured the use of MCP over the use of 2,4-D formulations, although the major weed species were more or less equally well controlled by each herbicide used. Unlike oats, the flax was injured by 2,4-D treatment at both growth stages but was consistently less tolerant at the later date of treatment. Since the bud stage of the major weed species and the flax occurred at nearly the same time the increased severity of the crop injury at the later treatment date might be explained. Aside from the effect on the yield of flax the 2,4-D esters delayed the maturity of this crop by 4 to 11 days. This is of major importance, especially in seasons of early fall frosts such as occurred at Melfort in 1954.

In these investigations conditions did not favour the incidence of a major infestation of Russian thistle, a species which thrives under droughty conditions. The failure of MCP to control this weed was pointed out earlier by Coupland (3) and Sexsmith (16) and was confirmed by the observations at Brandon in 1955. It might then follow that, if a heavy infestation of Russian thistle is encountered, the use of 2,4-D esters during the seedling stage would be justified in that the increased yield due to the removal of the weed competition would offset the herbicide injury to the crop.

The barley yields at Melfort in 1955 following the MCP and 2,4-D treatments were not sufficiently different to warrant the extra cost of the former product. This would support the work of Friesen and Olson (9), who found that barley was highly tolerant to 2,4-D after the 4-leaf stage was reached.

ACKNOWLEDGEMENTS

The authors wish to express their indebtedness to each of the following who co-operated in this investigation and contributed data: D. E. Forsberg, Experimental Farm, Scott, Sask.; A. Wenhardt and K. F. Best, Experimental Farm, Swift Current, Sask.; B. K. McDonald, Experimental Farm, Melfort, Sask.; H. W. Leggett and E. S. Molberg, Experimental Farm, Regina, Sask.; D. A. Brown, Experimental Farm, Brandon, Man., and W. J. Breakey, Experimental Farm, Morden, Man.

The authors also wish to express their appreciation to P. O. Ripley and K. W. Hill, of the Field Husbandry Division, Central Experimental Farm, Canada Department of Agriculture, Ottawa, for helpful suggestions in the progress of this work.

REFERENCES

1. Burrows, V. D., and P. J. Olson. Reaction of small grains to various densities of wild mustard and the results obtained after their removal with 2,4-D or by hand. I. Experiments with wheat. Can. J. Agr. Sci. 35 : 68-75. 1955.
2. Burrows, V. D., and P. J. Olson. Reaction of small grains to various densities of wild mustard and the results obtained after their removal with 2,4-D or by hand. II. Experiments with flax. Can. J. Agr. Sci. 35 : 193-201. 1955.
3. Coupland, R. T. Comparative effect of several herbicides on Russian thistle (Salsola pestifer A. Nels). Natl. Weed Comm. (Western Section) Research Rept. 1954.
4. Craig, H. A., D. J. Hill, and A. C. Arnal. The effect of 2,4-D and MCP on dog mustard and other weeds. Abst. Natl. Weed Comm. (Western Section) Research Rept. 1954.
5. Dunham, R. S. 2,4-D or MCP for weed control in flax, oats and legumes. Proc. North Central States Weed Control Conf. 11 : 119-120. 1954.
6. Friesen, H. A. Effect of 2,4-D on cereals. Proc. and Abstr., 3rd Western Canadian Weed Control Conf. 1949.
7. Friesen, H. A. The effect of herbicides on annual weeds growing on land sown to wheat and on land in fallow. Proc. and Abstr., 4th Western Canadian Weed Control Conf. 1950.
8. Friesen, H. A. The effect of various formulations of MCP and 2,4-D on weeds in grain crops. Proc. North Central Weed Control Conf. 1954.
9. Friesen, G., and P. J. Olson. The effect of 2,4-D on the developmental processes in barley and oats. Can. J. Agr. Sci. 33 : 315-329. 1953.
10. Leggett, H. W. The effect of 2,4-D sprayed on Larain oats at three day intervals. Proc. and Abstr. 4th Western Canadian Weed Control Conf. 1950.
11. Leggett, H. W. Effect of MCP on hemp nettle. Expt. Farm, Lacombe, Alberta, Annual Rpt. (*unpublished*). 1953.
12. McDonald, B. K. 2,4-D and MCP formulations for the control of annual weeds in flax. Natl. Weed Comm. (Western Section) Research Rpt. (Abstr.). 1954.
13. Olson, P. J., Saul Zalik, W. J. Breakey, and D. A. Brown. Sensitivity of wheat and barley at different stages of growth to treatment with 2,4-D. Agron. J. 43 : 77-83. 1951.
14. Olson, P. J. Effects of herbicides on cereals with special reference to oats. Proc. Joint Meeting of North Central Weed Control Conf. and Western Canadian Weed Control Conf. 1952.
15. Shebeski, L. H. Weed competition as affected by the time of spraying. Proc. of 8th Western Canadian Weed Control Conf. 1955.
16. Sexsmith, J. J. Effects of late application of several systemic herbicides to Russian thistle in flax. Research Rept., Nat. Weed Comm. (Western Canada Section). 1954.
17. Tandon, R. K. Response of flax varieties to rates and formulations of 2,4-D. Agron. J. 41 : 5. 1949.

FURTHER NOTE ON SURFACTANTS IN CONCENTRATE MIXTURE FOR CONTROL OF APPLE SCAB

Provided a surfactant is added to a concentrate spray, the effectiveness of insecticides and acaricides is not materially affected if the volume of spray is reduced to 50 gallons per acre. Advantages of low-volume sprays include a reduction in time required for spraying and a decrease in the amount of spray material which will accumulate on lower parts of fruit and on tips of leaves.

Experiments were undertaken at the Entomological Laboratory, Summerland, B.C., to determine if apple scab could be controlled as effectively with 50 gallons as with 75 gallons of spray per acre. The effect of added surfactants was also determined, as it had been demonstrated previously (1) that apple scab could be controlled with the 75-gallon-per-acre application when a surfactant was included in the spray.

The orchards (Sewell and Fransen) and sprayer were those used in 1954 (1). Five plots were laid out in each orchard, one plot serving as non-sprayed check. Speed of travel was one m.p.h., and spray output was regulated by nozzle orifice. Toxicants were applied at equal rates to all sprayed plots. These toxicants were: lime sulphur in four pre-cover sprays, ferbam and wettable sulphur in the first and second cover sprays, and ziram in the third cover spray.

By mid-July non-sprayed check trees were almost defoliated, and the fruit was small and severely infected with apple scab. Infection was evident also on fruit and newly developed terminal foliage in sprayed plots. In order to obtain a high degree of infection, for adequate comparison of treatments, the spray normally applied at this time was omitted and a final cover spray applied 3 weeks later, on August 6.

Table 1 shows the incidence of scab in 2,000 apples picked at random from each plot. The addition of Triton B-1956 to the spray mixtures improved scab control with both spray volumes. Seventy-five gallons

TABLE 1.—SCAB INFECTION IN PLOTS IN TWO ORCHARDS

Plot	Volume of spray applied (gal./acre)	Surfactant[1]	Scabby fruit	
			Sewell (per cent)	Fransen (per cent)
1	75	added	26.2	[2]
2	75	none	42.5	18.5
3	50	added	37.3	15.0
4	50	none	61.4	47.4
5	none	none	100.0	100.0

[1] One quart Triton B-1956 per 100 gal. (Rohm and Haas Co., Philadelphia, Pa.).
[2] Results unreliable owing to foaming of third cover spray.

appeared to be superior to the lower volume, but 50 gallons with surfactant was as effective as 75 gallons without surfactant.

REFERENCE

1. Swales, J. E., and K. Williams. Non-ionic surfactants in concentrate mixtures for the control of apple scab. Can. J. Agr. Sci. 36 : 36-40. 1956.

— J. E. SWALES
District Horticulturist,
British Columbia
Department of Agriculture,
Creston, B.C.
— K. WILLIAMS
Chemistry Division, Canada
Department of Agriculture,
Summerland, B.C.

September 10, 1956

Change in Publication Policy

The publication of the *Canadian Journal of Agricultural Science* in its present form terminated with the November-December, 1956, issue. It is being replaced by three new journals, namely:

Canadian Journal of Plant Science,

Canadian Journal of Soil Science,

Canadian Journal of Animal Science.

The *Canadian Journal of Plant Science* will be published in the months of January, April, July and October.

The *Canadian Journal of Soil Science* will be published in the months of February and August.

The *Canadian Journal of Animal Science* will be published in the months of June and December.

Publication of the new journal series will commence January, 1957, and the volume numbers for each new journal will follow the last volume of the *Canadian Journal of Agricultural Science*, i.e., each new journal to commence with Volume 37.

Subscription rates for the new journals will be as follows:

	Canada, U.S.A., Mexico, Central and South America, and British Commonwealth	*Other Countries*
Canadian Journal of Plant Science	**$4.00**	**$5.00**
Canadian Journal of Soil Science	**2.00**	**2.50**
Canadian Journal of Animal Science	**2.00**	**2.50**

The price of single numbers of all journals will be $1.25.

Present subscriptions to the *Canadian Journal of Agricultural Science* will be honoured to expiry dates.

Correspondence and manuscripts should be addressed to the appropriate journal at:

AGRICULTURAL INSTITUTE OF CANADA,
338 Somerset Street West,
Ottawa 4, Canada.